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Bioorganic & Medicinal Chemistry 14 (2006) 6759–6777
Bioorganic & Medicinal Chemistry

Synthesis of lipid A monosaccharide analogues containing acidic amino acid: Exploring the structural basis for the endotoxic and antagonistic activities

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Received 23 March 2006; revised 25 May 2006; accepted 25 May 2006
Available online 7 July 2006

Abstract—For elucidation of the structural and conformational requirements on the endotoxic and antagonistic activity of lipid A derivatives, we designed and synthesized lipid A analogues containing acidic amino acid residues in place of the non-reducing end phosphorylated glucosamine. Definite switching of the endotoxic or antagonistic activity was observed depending on the difference of the acidic groups (phosphoric acid or carboxylic acid) in the lipid A analogues.

1. Introduction

The innate immune system is the first line of the host defense against invading microorganisms. It is a phylogenetically ancient mechanism found in essentially every multicellular organism from plants to humans.[1] Toll-like receptors (TLRs) play a fundamental role in the molecular recognition of microbial components, such as lipopolysaccharide (LPS), peptidoglycan (PGN), lipoprotein, bacterial DNA, and viral RNA.[2,3] LPS is sensed by a receptor complex consisting of TLR4[4–8] and an adaptor protein MD-2.[9,10] LPS is a component of the outer membrane of Gram-negative bacteria, and is known as a potent immunostimulator or endotoxin for its strong activity which sometimes causes lethal sepsis.[11–15] It was previously demonstrated in our laboratory that the terminal glycolipid part, lipid A, is the chemical entity responsible for the biological activity of LPS by our total synthesis of *Escherichia coli* lipid A (**1**),[16,17] which consists of a glucosamine disaccharide having two phosphate groups and six acyl chains (Fig. 1). In contrast, the biosynthetic precursor **2** of LPS with four acyl chains shows antagonistic activity against cytokine induction stimulated with LPS in the human system (Fig. 1).[16,18–20]

Some monosaccharide lipid A analogues have been reported to date. The non-reducing sugar moiety (compound 411) of *E. coli* lipid A and most of its known analogues having saturated acyl chains showed cytokine (e.g., IL-8, TNF-α)-inducing activity.[21–23] In contrast, the reducing sugar moiety (compound 401, lipid X) **4** showed almost no activity for the cytokine induction,[21] but has weak antagonistic activity against LPS.[16,24]

The previous results on the structure–bioactivity relationships have shown the number and length of acyl chains and also the acidic groups are important for the bioactivity of lipid A.[25–28] The NMR conformation analysis of the tetraacylated lipid A **2** revealed that **2** takes a particular conformation and forms a characteristic supramolecular assembly.[29] These studies indicate that hydrophobic interaction between acyl groups is important for maintaining the conformation of lipid A, which would be recognized by LPS receptors.

Hawkins reported weak LPS receptor agonists that have a flexible backbone without the disaccharide structure.[30] Their study indicated that the disaccharide backbone is

Keywords: Innate immunity; Lipid A; Cytokine induction; IL-6; Endotoxin.
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doi:10.1016/j.bmc.2006.05.051

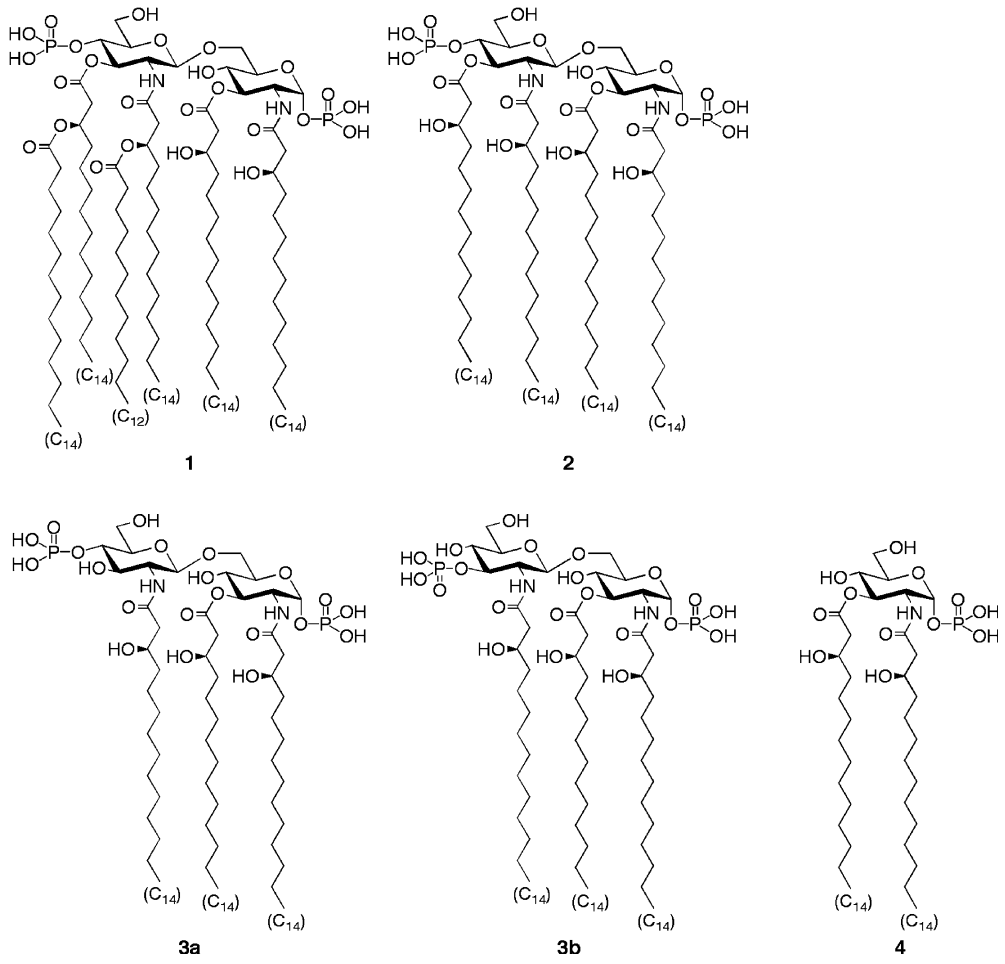


Figure 1. The chemical structures of *Escherichia coli* lipid A (506) **1**, its biosynthetic precursor (406) **2**, triacylated analogues of lipid A with 4′-phosphate group **3a** and with 3′-phosphate group **3b** and the right part of *E. coli* lipid A (lipid X) **4**.

not essential for the immunostimulatory activity but elucidation of the endotoxic conformation is difficult because of the conformational flexibility of their agonists. These results prompted us to design new lipid A analogues by molecular modeling on the basis of the molecular mechanics calculation, which was used for conformational analysis of **2**. The bioactive conformation such as the relative spatial position of two acidic moieties and the arrangement of the acyl groups can be estimated by evaluation of the biological activity of the analogues.

Recently, Miyake demonstrated that a potent LPS receptor agonist such as *E. coli* LPS and lipid A induced TLR4/MD-2 dimerization but antagonists did not. They also revealed that maximal binding of the antagonistic biosynthetic precursor analogue to TLR/MD-2 was 2-fold higher than that of the agonistic *E. coli* lipid A analogue, suggesting two TLR4/MD-2 bind to a single molecule of agonist **1**, whereas one TLR4/MD-2 binds to one antagonist **2**.[31,32] The size of the hydrophobic moieties should determine the response of TLR4/MD-2 but the structural difference between LPS agonists and antagonists is not clearly understood. Elucidation of the structural requirements for these activities is another objective of the present study.

In this study, we thus designed a series of lipid A analogues to investigate the structural requirements for the biological activities based on the triacyl-type lipid A analogue **3b** (Fig. 1),[25] which was synthesized in our group and revealed as an antagonist against LPS. The non-reducing end of lipid A (3-phosphorylated glucosamine) was substituted with an acidic amino acid such as aspartic acid or phosphoserine (Fig. 2), and the acylation arrangement was varied. We here synthesized 12 compounds of acidic amino acid-substituted lipid A analogues and observed the biological activities by such as the *Limulus* test and human cytokine (IL-6) induction assay.

2. Results and discussion

2.1. Design of the monosaccharide analogues of lipid A

In our previous study, we synthesized triacyl lipid A analogues with a 4′-phosphate group (**3a**) and 3′-phosphate group (**3b**) which have shown antagonistic activity against LPS.[25] Therefore, we have designed novel lipid A analogues based on **3b**, in which the non-reducing end (3-phosphorylated glucosamine) was substituted with aspartic acid or phosphoserine containing D- or L-configurations at the α-position of the amino acids,

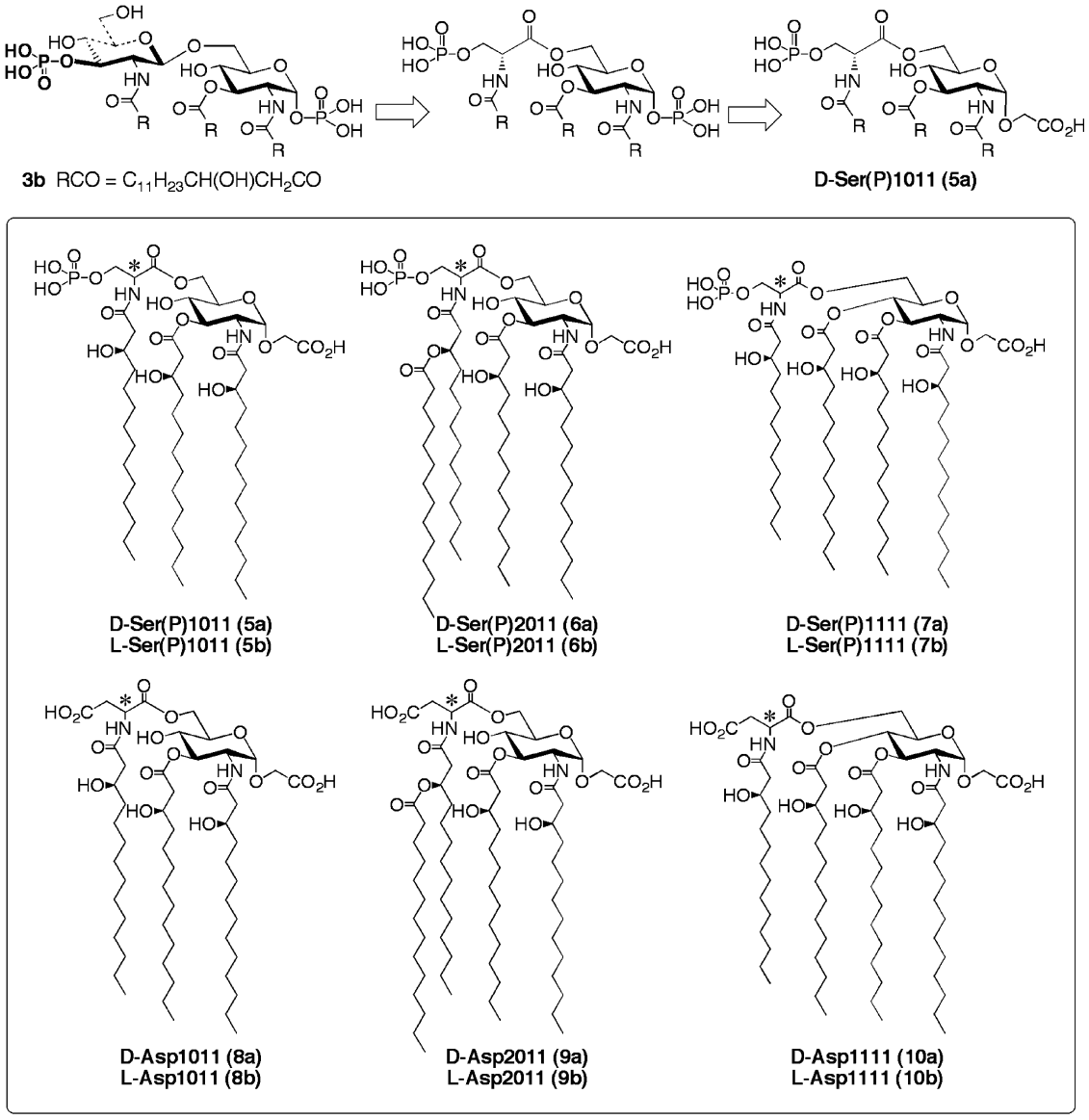


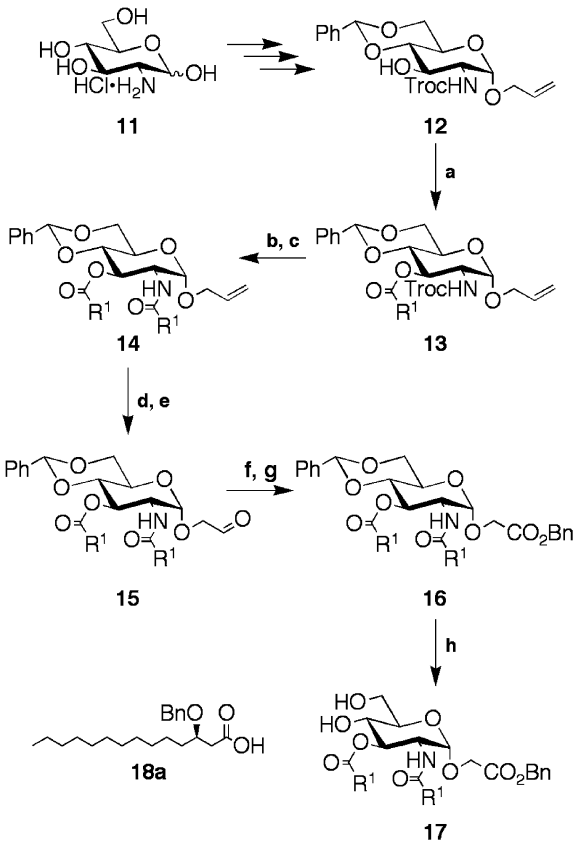
Figure 2. The structural design of the monosaccharide analogues of lipid A containing acidic *N*-acyl-amino acids.

and three types of acyl group distributions were introduced (Fig. 2). As it was also previously shown that the 1-phosphate group can be replaced with a carboxymethyl group without loss of the activity in the case of *E. coli* type lipid A (506) and the biosynthetic precursor,[33,34] we thus used a more stable and synthetically easy carboxymethyl group at the 1-position of glucosamine.

To predict conceivable conformations of each molecule in water, the lower energy conformations of 12 newly designed monosaccharide analogues of lipid A **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, **10a**, and **10b** in water were calculated by using the united atom AMBER* forcefield. The results showed that all analogues tend to gather the acyl chains to form a large hydrophobic domain as in lipid A (506) **1** or a biosynthetic precursor (406) **2**,[29] though the relative spatial position of two acidic moieties and acyl groups was different depending on the acylation pattern.

2.2. Synthesis of the monosaccharide analogues of lipid A

For the synthesis of monosaccharide lipid A analogues containing an acidic *N*-acyl-amino acid, aspartic acid or phosphoserine, we first synthesized the key intermediate **17** as shown in Scheme 1. First, the D-glucosamine derivative **12** possessing a free hydroxyl group at the 3-position was prepared from D-glucosamine hydrochloride **11**.[35] Condensation of **12** and (*R*)-3-benzyloxytetradecanoic acid **18a** with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 gave **13** in 91% yield. The trichloroethoxycarbonyl (Troc) group at the 2-position was reductively cleaved with a Zn–Cu couple in acetic acid, followed by introduction of **18a** to obtain glycolipid **14** in 86% yield for 2 steps. The anomeric allyl group of **13** was oxidized with osmium (VIII) oxide to afford a diol and successive oxidative cleavage with lead (IV) acetate gave the aldehyde **15** in 84% yield from **14**. Further oxidation with sodium chlorite and 2-methyl-2-butene under phos-



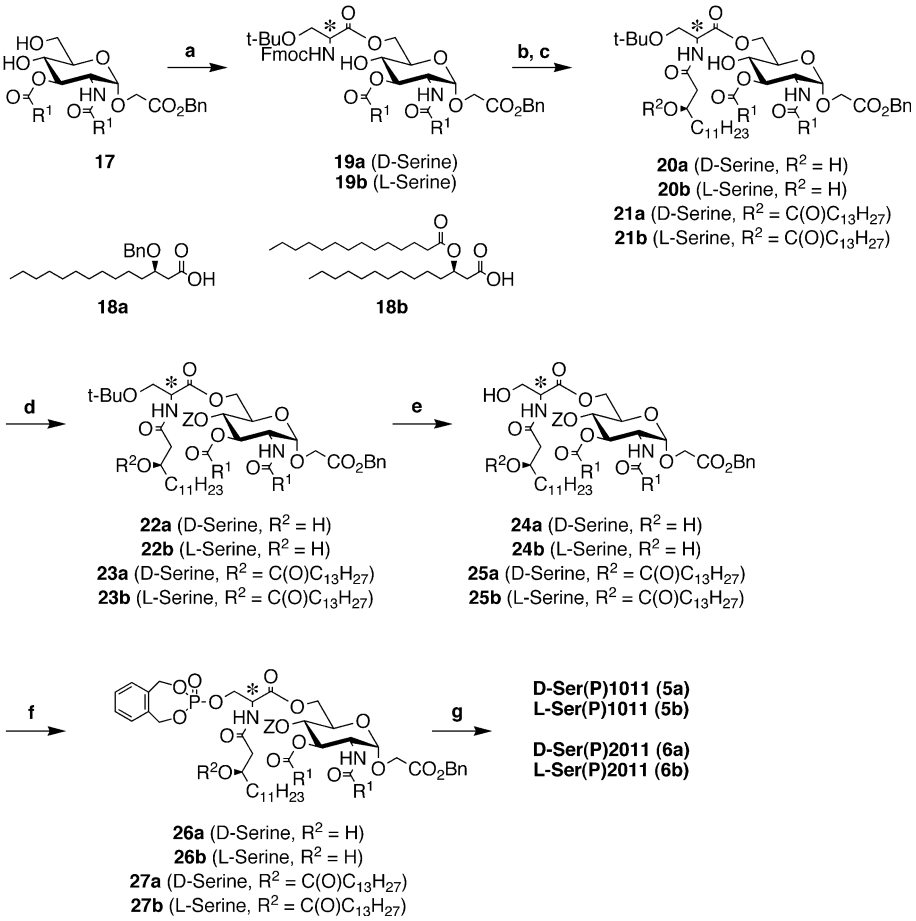
Scheme 1. Reagents and conditions: (a) **18a**, DCC, DMAP, CH_2Cl_2, 91%; (b) Zn–Cu couple, AcOH; (c) **18a**, WSCD·HCl, HOAt, CH_2Cl_2, 86% from **13**; (d) OsO_4, *N*-methylmorpholine-*N*-oxide, THF, *t*-BuOH, H_2O; (e) $Pb(OAc)_4$, benzene, 84% from **14**; (f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, THF, *t*-BuOH, H_2O; (g) $PhCHN_2$, CH_2Cl_2, 0 °C, 89% from **15**; (h) 90% AcOH, 50 °C, quant.

phate-buffered solution yielded a 1-*O*-carboxymethyl compound, which was then protected with a benzyl group by treatment with phenyldiazomethane to give **16** in 89% yield over 2 steps from **15**. Cleavage of the 4,6-*O*-benzylidene group was quantitatively achieved by heating up to 50 °C in 90% acetic acid solution to give a common intermediate **17**.

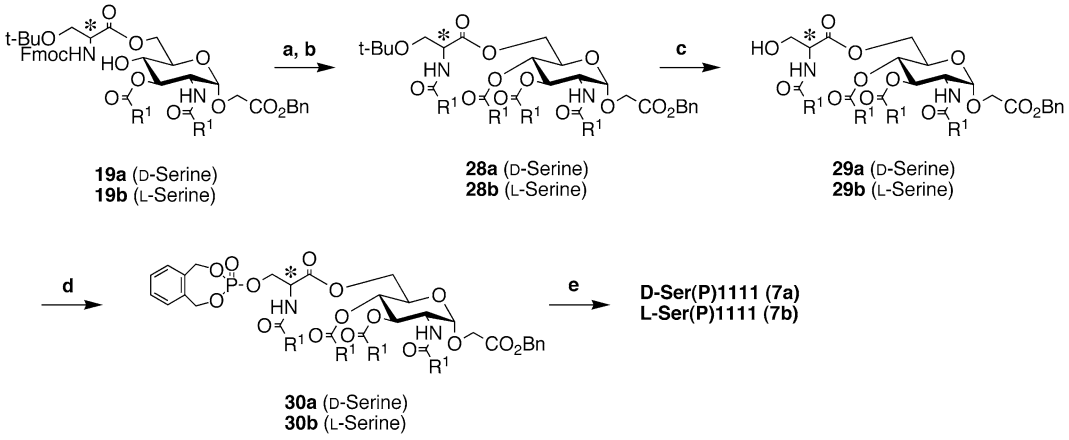
As shown in Scheme 2, an *N*-9-fluorenylmethoxycarbonyl (Fmoc)-protected serine derivative, Fmoc-D-Ser(*t*Bu) or Fmoc-L-Ser(*t*Bu), was introduced to the 6-hydroxyl group of monosaccharide **17** by using 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) and *N*-methylimidazole (NMI) in THF to give **19a** (D-serine) or **19b** (L-serine), respectively.[36] The Fmoc groups of **19a** and **19b** were cleaved with TBD (1,3,5-triazabicyclo[4.4.0]dec-5-ene)-methyl polystyrene[37] resin to facilitate the work-up operation only by filtration. Then, the free amino group of the serine residue was acylated with (*R*)-3-benzyloxytetradecanoic acid **18a** for **20a** and **20b**, or with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid **18b** for **21a** and **21b**. In the next step, the 4-hydroxyl group of **20a**, **20b**, **21a**, and **21b** was protected. An attempt for the protection with a benzyl group by treatment with benzyl bromide and silver oxide was unsuccessful presumably because of the low reactivity of the hydroxy group due to steric hindrance. We then tried benzyloxycarbonylation with 1-(benzyloxycarbonyl)benzotriazole (ZOBt) to obtain **22a**, **22b**, **23a**, and **23b**, respectively. Treatment with trifluoroacetic acid (TFA) in anhydrous dichloromethane to cleave the *tert*-butyl group of the serine residue unexpectedly led to hydrolysis of ester at the 6-position of glucosamine. This side reaction was prevented by adding anisole as a scavenger to yield **24a**, **24b**, **25a**, and **25b** in the range of 80% to quantitative yields. Subsequent phosphorylation was successfully achieved with *o*-xylylene-*N*,*N*-diethylphosphoramidite (XEPA) and 1*H*-tetrazole, followed by oxidation with *m*-chloroperbenzoic acid (mCPBA) to give **26a**, **26b**, **27a**, and **27b** in 40–59% yields.[38] Finally, catalytic hydrogenolysis afforded the desired products **5a** (D-**Ser(P)1011**), **5b** (L-**Ser(P)1011**), **6a** (D-**Ser(P)-2011**), and **6b** (L-**Ser(P)2011**), respectively.

Compounds **7a** (D-**Ser(P)1111**) and **7b** (L-**Ser(P)1111**) were synthesized as shown in Scheme 3. First, the Fmoc groups of the serine of **19a** and **19b** were cleaved with TBD–methyl polystyrene, and subsequent condensation with two equivalents of fatty acid **18a** to both the amino group at the serine residue and the hydroxyl group at the 4-position of glucosamine simultaneously gave compounds **28a** and **28b** by using 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl) and 1-hydroxy-7-benzotriazole (HOBt) together with DMAP. After cleavage of the *tert*-butyl group with TFA, introduction of a phosphate moiety to the resulting **29a** and **29b** gave **30a** and **30b**. Then, subsequent hydrogenolysis gave **7a** (D-**Ser(P)1111**) and **7b** (L-**Ser(P)1111**).

Six kinds of aspartic acid-substituted analogues **8a**, **8b**, **9a**, **9b**, **10a**, and **10b** were also synthesized with the procedures (Scheme 4) similar to the synthesis of phosphoserine-substituted analogues **5a**, **5b**, **6a**, and **6b**. In this synthesis, commercially available aspartic acid derivatives, Boc-D-Asp(OBn) and Boc-L-Asp(OBn), were condensed to the monosaccharide **17** to give **31a** and **31b**, respectively. In this condensation, no racemization at the chiral center of aspartic acid was observed. The *tert*-butoxycarbonyl (Boc) groups of compounds **31a** and **31b** were cleaved under acidic conditions, then each fatty acid, (*R*)-3-benzyloxytetradecanoic acid **18a** or (*R*)-3-(tetradecanoyloxy)tetradecanoic acid **18b**, was condensed to the liberated amines to obtain **32a**, **32b**, **33a**, and **33b**. These compounds were catalytically hydrogenated in THF under 20 kgf/cm^2 of H_2 atmosphere to yield **Asp1011**s (**8a** and **8b**) and **Asp2011**s (**9a** and **9b**). In the synthesis of **Asp1111**s (**10a** and **10b**), after deprotection of the Boc group in **31a** or **31b**, two equivalents of **18a** was condensed to both the liberated amine at the aspartic acid residue and the hydroxyl group at the 4-position of glucosamine. Finally, palladium-catalyzed hydrogenolysis cleaved all the benzyl-type protecting groups to yield the desired compounds, D-**Asp1111** (**10a**) and L-**Asp1111** (**10b**). The monosaccharide part of the analogues (**35**) was also synthesized by hydrogenolysis of **17** with palladium-black catalyst (Scheme 5). We thus achieved the synthesis of 12 analogues and applied them to a biological study.



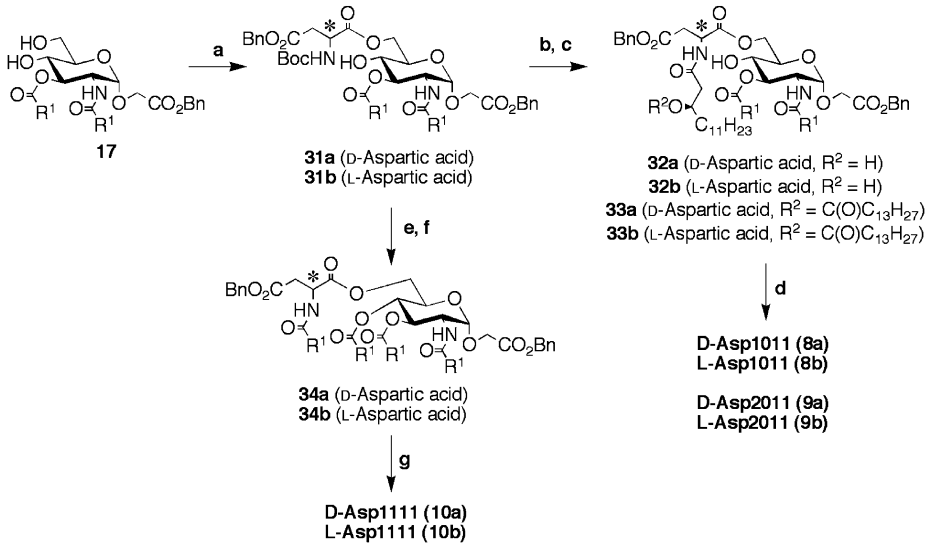
Scheme 2. Reagents and conditions: (a) Fmoc-D-Ser(^{t}Bu) or Fmoc-L-Ser(^{t}Bu), 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole, *N*-methylimidazole, THF, 85–91%; (b) TBD–methyl polystyrene, THF; (c) **18a** or **18b**, WSCD·HCl, HOBt, CH_2Cl_2, 52–68%; (d) ZOBt, DMAP, THF, 50–60 °C, 80%–quant.; (e) trifluoroacetic acid, anisole, CH_2Cl_2, 80%–quant.; (f) XEPA, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −20 °C, 40–59%; (g) palladium-black, H_2 (20 kgf/cm^2), THF, 74%–quant.



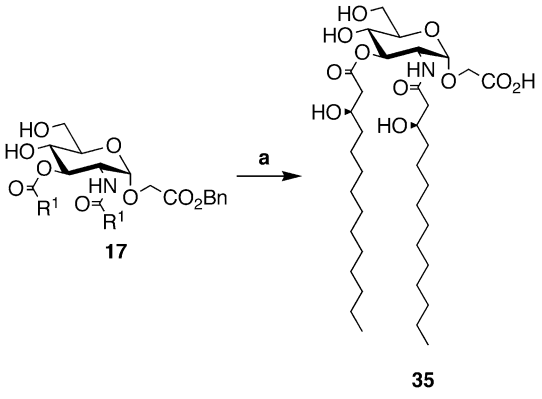
Scheme 3. Reagents and conditions: (a) TBD–methyl polystyrene, THF; (b) **18a**, WSCD·HCl, HOBt, DMAP, CH_2Cl_2, 55–61%; (c) trifluoroacetic acid, anisole, CH_2Cl_2, 82–83%; (d) XEPA, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −20 °C, 49–81%; (e) palladium-black, H_2 (20 kgf/cm^2), THF, 67–93%.

2.3. Biological study

For detection of LPS, the *Limulus* test has been often used. This test uses the reaction of *Limulus* amebocyte lysate (LAL) of horseshore crab for quantitative measurement of LPS.[39,40] We carried out the test and the result showed the *Limulus* activities of our synthetic analogues were obviously affected by the distribution of the acyl chains (Fig. 3). The 2011-type compounds (marked with circle) showed strongest *Limulus* activities in three acylation patterns among the same amino acid-substituted analogues. The 1011-type compounds



Scheme 4. Reagents and conditions: (a) Boc-D-Asp(OBn) or Boc-L-Asp(OBn), DCC, DMAP, CH_2Cl_2, 73–97%; (b) trifluoroacetic acid, CH_2Cl_2; (c) (*R*)-3-benzyloxytetradecanoic acid **18a** or **18b**, WSCD·HCl, HOBt, CH_2Cl_2, 64–77%; (d) palladium-black, H_2 (20 kgf/cm^2), THF, 82%–quant.; (e) trifluoroacetic acid, CH_2Cl_2; (f) **18a**, WSCD·HCl, HOBt, CH_2Cl_2, 47–56%; (g) palladium-black, H_2 (20 kgf/cm^2), THF, 96–98%.



Scheme 5. Reagents: (a) palladium-black, H_2 (20 kgf/cm^2), THF, quant.

(triangle) showed comparatively lower but still showed definite activities, and 1111-type compounds (square) showed little activities.

We also measured human cytokine induction and the inhibitory activities against LPS. Cytokine-inducing and inhibitory activities of the analogues and standard LPS (*E. coli* O111:B4) were tested in human peripheral whole-blood (hpwb) cells.[41] The stimulated levels of cytokines, that is, interleukin-6 (IL-6), were measured by means of enzyme-linked immunosorbent assay (ELISA). The IL-6-inducing activities (A) and the inhibitory activities (B) are shown in Figure 4. Most of analogues exhibited no induction of IL-6, but tetraacylated D-**Asp2011** (**9a**) exhibited concentration-dependent induction at the level over 100 ng/mL. L-**Asp2011** (**9b**) also exhibited the IL-6 induction at 10 μg/mL concentration, although the activity was about a 100-fold weaker than that of D-**Asp2011** (**9a**). In this assay, the monosaccharide analogue without amino acid (the reducing end part) **35** induced no IL-6 (Fig. 4A). As for the inhibitory activity against IL-6 induction by LPS, most of the analogues exhibited stronger inhibitory activities than the reducing end part **35**, whereas D-**Asp2011** (**9a**) and D-**Asp1011** (**8a**) did not show the activity (Fig. 4B). The phosphoserine-containing analogues showed stronger inhibitory activities than the corresponding aspartic acid-containing analogues having the same acylation pattern in general. Obvious trends were also observed between the inhibitory activities and the acylation patterns. In both cases of D- and L-phosphoserine analogues, the 2011-acylation form exhibited the highest inhibitory activity. The 1011-form showed slightly weaker activity than the 2011-form but stronger activity than the 1111-form. In the case of aspartic acid analogues, the L-form showed stronger inhibition than the D-form, while D- and L-phosphoserine analogues did not exhibit definite differences. The weaker activity of 1111-form might be caused by the difference of the relative position of two acidic moieties and acyl groups in 1111-form from that in 2011-form or 1011-form. Molecular mechanics calculations of D-Asp1011 (**8a**), D-Asp2011 (**9a**), and D-Asp1111 (**10a**) showed that **8a** and **9a** have similar spatial arrangement of acidic moieties and acyl groups but **10a** has the different spatial arrangement (Fig. 5). Probably, the 4-acyl group influences the packing of acyl chains and molecular conformation.

3. Conclusion

In the present study, we synthesized acidic amino acid-containing monosaccharide lipid A analogues with aspartic acid and phosphoserine having three kinds of acylation patterns. All the phosphoserine analogues showed the antagonistic activity, whereas D-**Asp2011** and L-**Asp2011** showed the immunostimulating activity. The agonistic and antagonistic activities were switched by structural change between the phos-

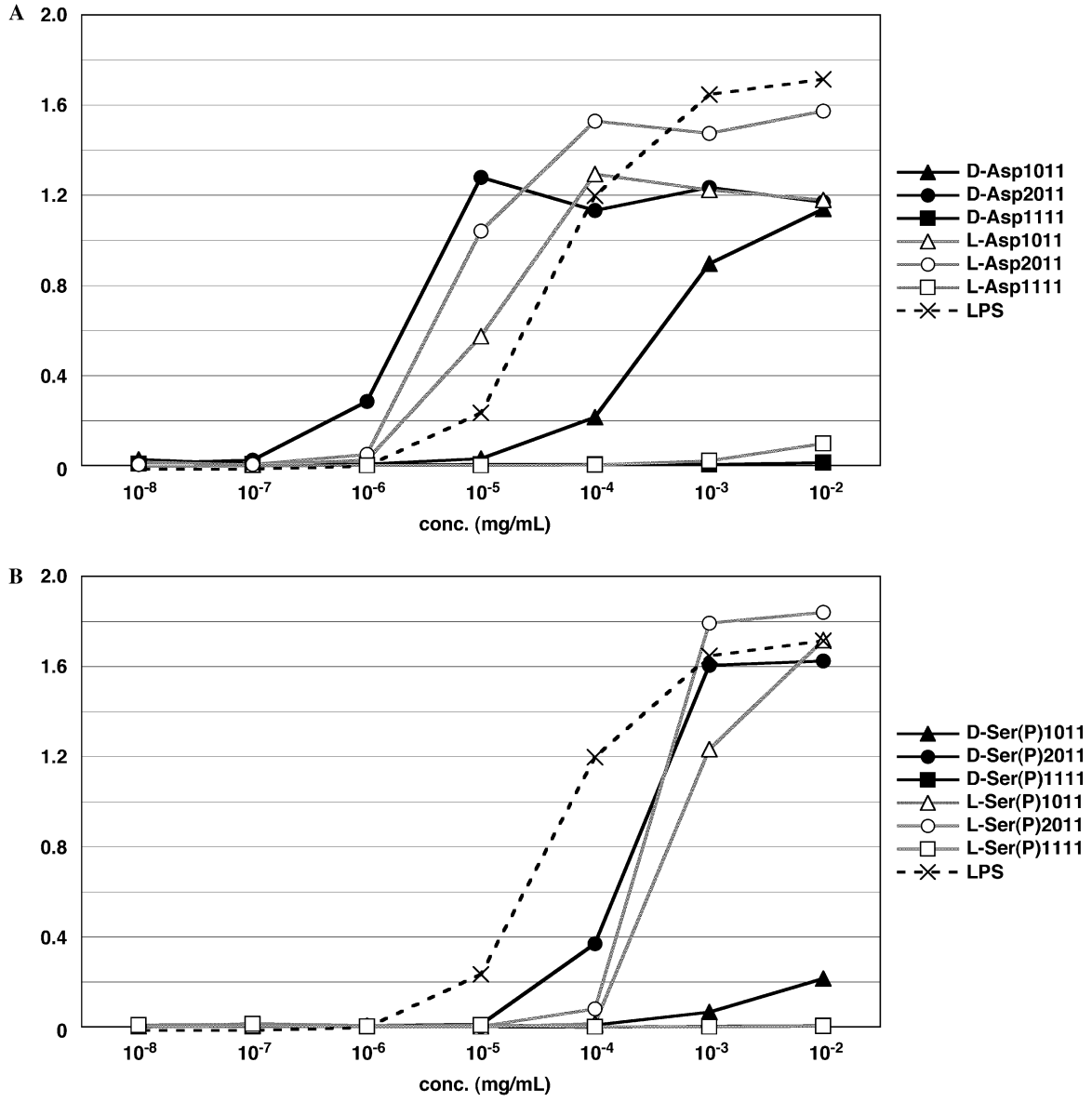


Figure 3. *Limulus* activities of synthetic lipid A analogues and LPS. (A) Aspartic acid-substituted analogues. (B) Phosphoserine-substituted analogues.

phoryl group (e.g., D-**Ser(P)2011**, **6a**) and the carboxylic group (D-**Asp2011**, **9a**). We also observed that the tetraacylated analogues showed stronger inhibitions than the triacylated analogues. The results suggested that the receptor protein of LPS/lipid A recognizes second acidic groups on amino acid residues, and also it seemed that the volume and shape of the hydrophobic part consisted with acyl chains were recognized by the receptor. In the case of *Limulus* test, the tendency was dramatically influenced by the acylation patterns. The present study clearly showed that structural requirements for expression of the *Limulus* activity are different from cytokine-inducing activities or its antagonistic activities, since 1111-type analogues did not stimulate *Limulus* test. We are now proceeding to investigate more detailed biological activities and the recognition mechanism by using the monosaccharide lipid A analogues.

4. Experimental

4.1. General methods

NMR spectra were measured with a JEOL JNM-GX400 and a JEOL JMM-GX270 at 30 °C unless otherwise specified, and analyzed using Alice® program (version 2.0). The proton chemical shifts in $CDCl_3$ are given in δ values from tetramethylsilane as an internal standard, and the chemical shifts in other solvents or conditions are given in δ values from the residual proton signal of the solvent. Mass spectra were obtained with ESI-TOF mass spectrometer (Applied Biosystems, Mariner™). Specific optical rotations were measured on a Perkin-Elmer 241 polarimeter. Recycling preparative HPLC was carried out with Japan Analytical Industry LC908. Silica-gel chromatography was performed using Kieselgel 60 (Merck, 0.040–0.063 mm) under medium

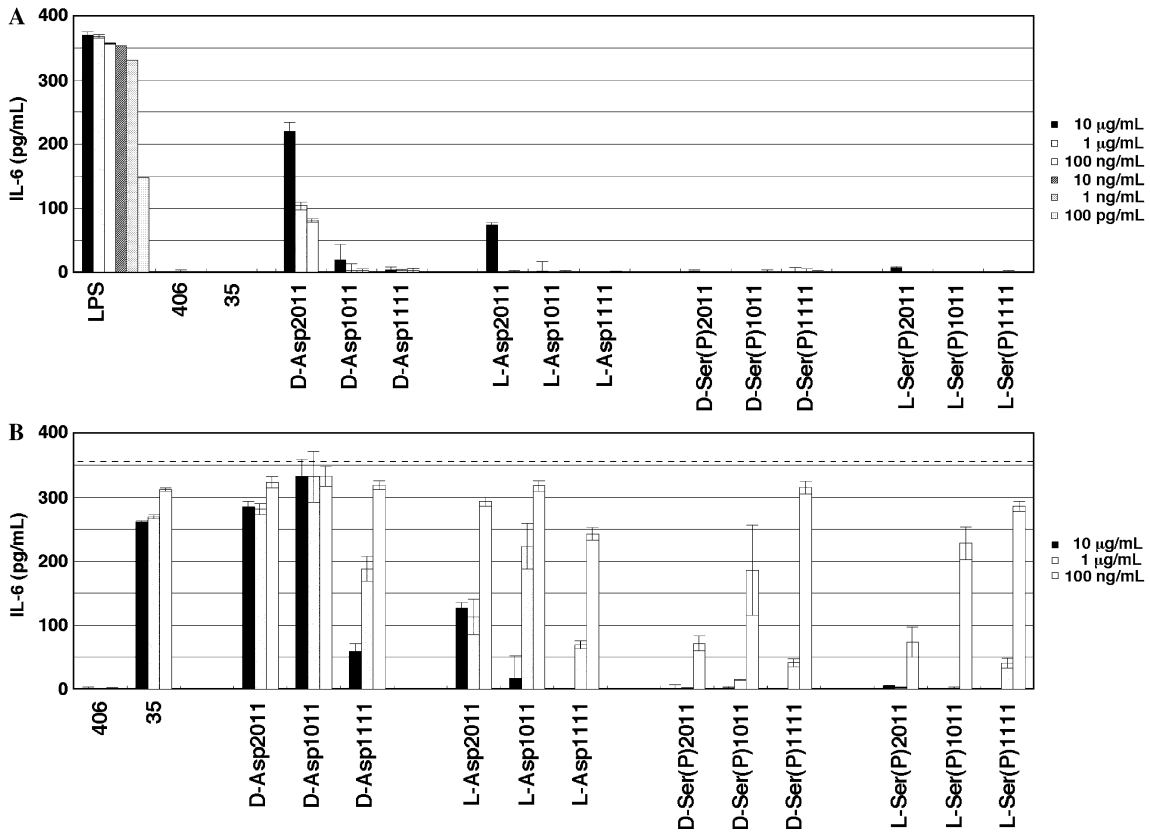


Figure 4. (A) IL-6 induction activity of synthetic lipid A analogues in comparison with LPS (*Escherichia coli*, O111:B4). (B) Antagonistic activity of synthetic lipid A analogues to suppress IL-6 induction by LPS (*Escherichia coli*, O111:B4, 5 ng/mL). The induced level of IL-6 was determined by ELISA. Average values of three repeated assays and deviations of individual experiments are given in the graph. In B, the dashed line shows the level of the IL-6 induction of positive control (5 ng/mL of LPS).

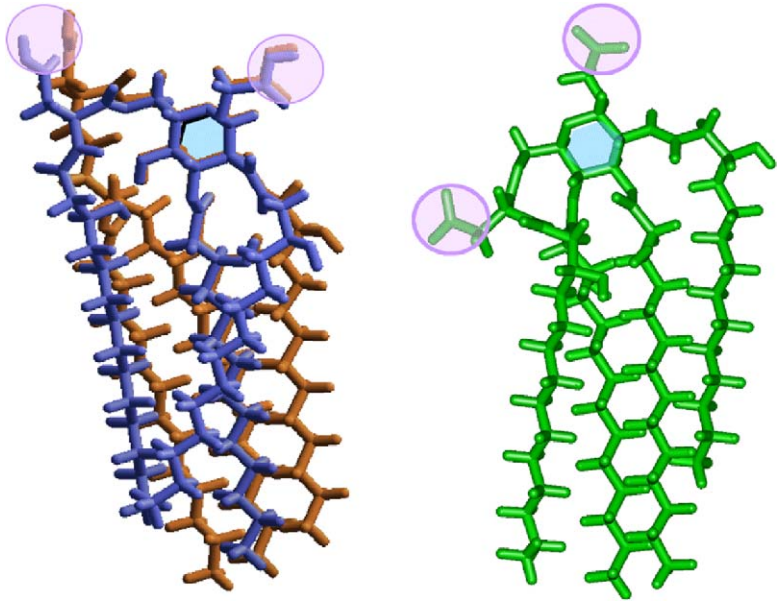


Figure 5. The lower energy conformations of D-Asp2011 (brown), D-Asp1011 (blue), and D-Asp1111 (green), calculated by using the united atom AMBER* forcefield, with MontecCarlo-Low mode conformational search. Sugar moieties (light blue) and acidic moieties (pink) were highlighted.

pressure (2–4 kg/cm^2) using the indicated solvent systems. Analytical and preparative thin-layer chromatographies (TLC) were performed on Kieselgel 60F_{254} Plates (Merck, 0.25 mm thickness) and precoated Kieselgel 60F_{254} Plates (Merck, 0.5 mm thickness), respectively. Nonaqueous reactions were carried out under argon atmosphere unless otherwise noted. Anhydrous dichloromethane (CH_2Cl_2) was prepared by distillation

from calcium hydride and phosphorus pentoxide. Anhydrous acetonitrile, tetrahydrofuran (THF), and toluene were purchased from Kanto Chemicals Co. Distilled water was purchased from Otsuka (Tokyo, Japan) or prepared by a combination of Toray Pure LV-308 (Toray) and GSL-200 (Advantec, Tokyo, Japan). The water was used as an eluent of liquid–liquid partition column chromatography and a solvent of lyophilization. Molecular sieves 4 Å were activated in vacuo at 250 °C for 3 h before use. All other commercially obtained materials were used as received.

4.2. Computational analysis

To predict the conformation of our synthetic compound in water, we carried it out with the united atom Amber* forcefield in the environment of generalized Born/surface area (GB-SA) continuum solvent model for water. The molecular mechanics methods and conformational search (MCMM (Monte Carlo Multiple Minimum) and Low Mode calculations) calculation of lipid A analogues were performed with MacroModel™ 8.1 (Schrödinger Inc.).

4.3. Biological assay

We used commercially available standard LPS (*E. coli* O111:B4, Sigma Chemical Co.) for each biological assay. Biosynthetic precursor **2** used in this study was synthesized in our laboratory.[18,42]

Limulus test was carried out using the Endospacy™ ES-50M set (Seikagaku Kogyo, Tokyo, Japan). We performed the test according to the procedure included in the kit.

In cytokine induction assay, a mixture of a test sample and heparinized hpwb in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) was incubated at 37 °C in 5% CO_2 for 24 h. Otherwise, in cytokine inhibitory assay, a mixture of test sample, LPS (5 ng/mL), and heparinized hpwb was incubated and the level of cytokines induced was measured in the same manner.

4.4. Allyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (13)

To a solution of **12** (6.47 g, 13.0 mmol) and (*R*)-3-benzyloxytetradecanoic acid (**18a**) (5.38 g, 15.6 mmol) in absolute CH_2Cl_2 (100 mL) were added DMAP (318 mg, 2.6 mmol) and DCC (4.02 g, 19.5 mmol) at room temperature under N_2 atmosphere. After stirring for 2 h, the mixture was quenched with saturated aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$. The combined extracts were washed with aqueous 10% citric acid, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was suspended with AcOEt and the insoluble materials were removed. The crude sample was purified by silica-gel column chromatography (500 g, toluene/AcOEt = 50:1) to give **13** as a white solid (9.20 g, 89%). $[\alpha]_D^{23}$ +0.358 (*c* 1.0, $CHCl_3$); ESI-TOF (positive) *m*/*z* = 822.3 $[(M+Na)^+]$; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.42–7.21 (m, 10H, OCH_2Ph, *Ph*CH), 5.95–5.85 (m, *J* = 17.1 Hz, *J* = 10.4 Hz, *J* = 5.3 Hz, 1H, $OCH_2CH{=}CH_2$), 5.47 (s, 1H, PhC*H*), 5.44–5.39 (dd, *J* = 10.1 Hz, *J* = 9.9 Hz, 1H, H-3), 5.37–5.34 (d, *J* = 10.1 Hz, 1H, N*H*), 5.34–5.29 (dd, *J* = 17.2 Hz, *J* = 1.5 Hz, 1H, $OCH_2CH{=}CH_2$), 5.26–5.23 (dd, *J* = 10.4 Hz, *J* = 1.5 Hz, 1H, $OCH_2CH{=}CH_2$), 4.94–4.93 (d, *J* = 3.7 Hz, 1H, H-1), 4.74–4.71 (d, *J* = 12.1 Hz, 1H, OCH_2Ph), 4.60–4.57 (d, *J* = 12.1 Hz, 1H, OCH_2Ph), 4.52–4.49 (d, *J* = 12.1 Hz, 1H, CH_2 of Troc), 4.41–4.38 (d, *J* = 12.1 Hz, 1H, CH_2 of Troc), 4.30–4.27 (m, *J* = 4.7 Hz, *J* = 10.2 Hz, 1 H, H-6a), 4.24–4.19 (dd, *J* = 12.7 Hz, *J* = 5.3 Hz, 1H, $OCH_2CH{=}CH_2$), 4.10–4.00 (m, *J* = 3.7 Hz, *J* = 10.1, *J* = 10.1 Hz, *J* = 12.8 Hz, *J* = 6.4 Hz, 2H, $OCH_2CH{=}CH_2$, H-2), 3.98–3.92 (ddd, *J* = 4.7 Hz, *J* = 9.9 Hz, 1H, H-5), 3.83–3.68 (m, 3H, $C^{\beta}H$ of acyl, H-6b, H-4), 2.70–2.64 (dd, *J* = 15.3 Hz, *J* = 6.4 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.46–2.41 (dd, *J* = 15.3 Hz, *J* = 6.0 Hz, 1H, $C^{\alpha}H_2$ of acyl), 1.53–1.41 (m, 2H, CH_2 of acyl), 1.32–1.18 (CH_2 of acyl), 0.90–0.86 (t, *J* = 7.2 Hz, 3H, CH_3 of acyl); Found: C, 60.61; H, 6.70; N, 1.74. Calcd for $C_{40}H_{54}O_9NCl_3$: C, 60.11; H, 6.81; N, 1.75.

4.5. Allyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (14)

To a solution of **13** (2.05 g, 2.57 mmol) in AcOH (10 mL) was added zinc–copper couple (ca. 8.00 g). The suspension was stirred at room temperature for 2 h. After the precipitate was filtered off, the filtrate was coevaporated in vacuo with toluene. To the residue was added saturated aqueous $NaHCO_3$ (20 mL) and then the mixture was extracted with AcOEt. The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo to give crude amine which was used for the following reaction without further purification.

To the solution of the amine (*R*)-3-benzyloxytetradecanoic acid (**18a**) (0.94 g, 2.82 mmol) and HOBt (0.35 g, 2.57 mmol) in absolute CH_2Cl_2 (25 mL) was added WSCD·HCl (1.48 g, 7.70 mmol) at room temperature under Ar atmosphere. After stirring overnight, the mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (80 g, toluene/AcOEt = 50:1) to give **14** as a white solid (2.01 g, 84%). $[\alpha]_D^{23}$ +0.363 (*c* 1.0, $CHCl_3$); ESI-TOF (positive) *m*/*z* = 962.7 $[(M+Na)^+]$; ^1H NMR (400 MHz, $CDCl_3$), δ = 7.42–7.20 (m, 15H, OCH_2Ph, *Ph*CH), 6.30–6.28 (d, *J* = 9.5 Hz, 1H, N*H*), 5.78–5.68 (m, 1H, $OCH_2CH{=}CH_2$), 5.46 (s, 1H, PhC*H*), 5.41–5.36 (dd, *J* = 10.1 Hz, *J* = 9.9 Hz, 1H, H-3), 5.22–5.17 (dd, *J* = 17.2 Hz, *J* = 1.5 Hz, 1H, $OCH_2CH{=}CH_2$), 5.14–5.10 (dd, *J* = 10.4 Hz, *J* = 1.4 Hz, 1H, $OCH_2CH{=}CH_2$), 4.80–4.79 (d, *J* = 3.7 Hz, 1H, H-1), 4.56–4.48 (m, *J* = 11.6 Hz, 3H, OCH_2Ph, OCH_2Ph), 4.43–4.36 (m, *J* = 3.7 Hz, *J* = 10.5, *J* = 9.6 Hz, *J* = 11.6 Hz, 2H, H-2, OCH_2Ph), 4.27–4.24 (dd, *J* = 4.7 Hz, *J* = 10.2 Hz, 1H, H-6a), 4.06–4.00 (dd, *J* = 12.7 Hz, *J* = 5.5 Hz, 1H,

$OCH_2CH{=}CH_2$), 3.94–3.88 (ddd, J = 9.8 Hz, J = 4.6 Hz, J = 9.8 Hz, 1H, H-5), 3.84–3.68 (m, 5H, $OCH_2CH{=}CH_2$, $C^{\beta}H$ of acyl, H-6b, H-4), 2.70–2.64 (m, J = 15.3 Hz, J = 6.3 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.44–2.39 (dd, J = 15.3 Hz, J = 6.1 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.37–2.27 (m, 2H, $C^{\alpha}H_2$ of acyl), 1.61–1.20 (m, 40H, CH_2 of acyl), 0.90–0.86 (m, 3H, CH_3 of acyl); Found: C, 73.81; H, 9.06; N, 1.50. Calcd for $C_{58}H_{85}O_9N$: C, 74.09; H, 9.11; N, 1.49.

4.6. Formylmethyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (15)

To a solution of **14** (126.4 mg, 0.134 mmol) in THF/*t*-BuOH/H_2O = 10:10:1 (20.1 mL) were added 4-methylmorpholine *N*-oxide (64.7 mg, 0.536 mmol) and aqueous OsO_4 (1 M, 26.9 mL, 26.9 mmol) at room temperature. After stirring for 8 h, to the mixture was added 10% aqueous $Na_2S_2O_3$. The mixture was extracted with AcOEt, washed with 10% aqueous $Na_2S_2O_3$, and concentrated in vacuo to give the crude diol, which was subjected to the following oxidation without further purification.

To the solution of the diol in anhydrous benzene (1.2 mL) was added $Pb(OAc)_4$ (93%, 76.8 mg, 0.161 mmol) at room temperature under N_2 atmosphere. After stirring for 1 h, the mixture was filtered with Celite and extracted with AcOEt. The combined extracts were dried over Na_2SO_4, and coevaporated with toluene three times. The residue was purified by silica-gel column chromatography (10 g, $CHCl_3$/acetone = 20:1) to give **15** as a white solid (109.2 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.41 (s, 1H, C*H*O), 7.37–7.23 (m, 15H, OCH_2Ph, CH*Ph*), 6.58–6.55 (d, J = 9.5 Hz, N*H*), 5.45–5.35 (m, 2H, C*H*Ph, H-3), 4.73–4.72 (d, J = 3.5 Hz, 1H, H-1), 4.58-4.37 (m, 5H, OCH_2Ph, H-2), 4.24–4.20 (dd, J = 4.9 Hz, J = 10.2 Hz, 1H, H-6a), 3.94–3.67 (m, 7H, H-5, CH_2 of formylmethyl, $C^{\beta}H$ of acyl, H-6b, H-4), 2.70–2.65 (dd, J = 14.8 Hz, J = 6.0 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.46–2.28 (m, 3H, $C^{\alpha}H_2$ of acyl), 1.47–1.18 (m, 40H, CH_2 of acyl), 0.90–0.86 (t, 6H, CH_3 of acyl).

4.7. Benzyloxycarbonylmethyl 4,6-*O*-benzylidene-3-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-2-α-D-glucopyranoside (16)

To a suspension of **15** (942.3 mg, 1.00 mmol), NaH_2PO_4 (120.0 mg, 1.0 mmol), and 2-methyl-2-butene (530 mL, 5.00 mmol) in *t*-BuOH/H_2O = 4:1 (10 mL) was added $NaClO_2$ (80% purity, 113.1 mg, 1.00 mmol) at room temperature. After stirring overnight, the mixture was neutralized with aqueous 1 M HCl and freeze-dried to give the crude carboxylic acid, which was subjected to the following reaction without further purification.

To the suspension of the carboxylic acid in CH_2Cl_2 (10 mL) was dropped the Et_2O solution of phenyl diazomethane (0.46 M) at room temperature until the suspension turned a pale red. The mixture was quenched with AcOH (90% purity, 1 mL) and extracted with AcOEt. The combined extracts were washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (40 g, toluene/AcOEt = 10:1) to give **16** as a white solid (885.6 mg, 84%). $[\alpha]_D^{23}$ +0.348 (*c* 1.0, $CHCl_3$); ESI-TOF (positive) m/z = 1070.7 [$(M+Na)^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40–7.21 (m, 20H, OCH_2Ph, *Ph*CH), 6.71–6.69 (d, J = 9.5 Hz, 1H, N*H*), 5.44 (s, 1H, PhC*H*), 5.43–5.38 (dd, J = 9.9 Hz, J = 10.1 Hz, 1H, H-3), 5.12 (s, 2H, $COOCH_2$Ph), 4.80–4.79 (d, J = 3.7 Hz, 1H, H-1), 4.59–4.47 (m, 3H, OCH_2Ph, OCH_2Ph), 4.45–4.39 (ddd, J = 3.7 Hz, J = 9.9 Hz, J = 9.5 Hz, 1H, H-2), 4.38–4.35 (d, J = 11.6 Hz, 1H, OCH_2Ph), 4.22–4.18 (dd, J = 4.9 Hz, J = 10.4 Hz, 1H, H-6a), 4.01–3.92 (m, 3H, 1-O–CH_2–CO_2–, H-6b), 3.85–3.78 (m, 2H, $C^{\beta}H$ of acyl), 3.74–3.67 (m, 2H, H-5, H-4), 2.70–2.64 (m, J = 15.1 Hz, J = 6.6 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.43–2.37 (m, J = 15.1 Hz, J = 5.8 Hz, 3H, $C^{\alpha}H_2$ of acyl), 1.48–1.43 (m, 40H, CH_2 of acyl), 0.90–0.86 (m, 6H, CH_3 of acyl); Found: C, 72.94; H, 8.59; N, 1.36; Calcd for $C_{64}H_{89}O_{11}N$: C, 73.32; H, 8.56; N, 1.34.

4.8. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (17)

To the suspension of **16** (56.0 mg, 53.4 μmol) in CH_2Cl_2/H_2O = 50:1 (0.25 mL) was added the CH_2Cl_2 solution of trifluoroacetic acid (10%, 250 mL) at room temperature under N_2 atmosphere. After stirring for 40 min, the mixture was quenched with aqueous saturated $NaHCO_3$ (1 mL) and extracted with AcOEt. The combined extracts were washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (5 g, $CHCl_3$/acetone = 10:1) to give **17** as a white solid (46.2 mg, 90%). $[\alpha]_D^{23}$ +0.342 (*c* 1.0, $CHCl_3$); ESI-TOF (positive) m/z = 982.7 [$(M+Na)^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44–7.20 (m, 15H, OCH_2Ph), 6.68–6.66 (d, J = 9.5 Hz, 1H, N*H*), 5.16–5.11 (dd, J = 10.7 Hz, J = 9.0 Hz, 1H, H-3), 5.11 (s, 2H, $COOCH_2$Ph), 4.79–4.78 (d, J = 3.7 Hz, 1H, H-1), 4.55–4.50 (m, 4H, OCH_2Ph), 4.32–4.26 (ddd, J = 3.7 Hz, J = 10.7 Hz, J = 9.5 Hz, 1H, H-2), 4.03–3.98 (d, J = 16.6 Hz, 1H, 1-O–CH_2–CO_2–), 3.97–3.93 (d, J = 16.6 Hz, 1H, 1-O–CH_2–CO_2–), 3.90–3.84 (m, 1H, $C^{\beta}H$ of acyl), 3.83–3.79 (m, 1H, $C^{\beta}H$ of acyl), 3.75–3.68 (m, 3H, H-5, H-6a, H-6b), 3.65–3.60 (dd, J = 9.3 Hz, J = 9.3 Hz, 1H, H-4), 2.64–2.59 (dd, J = 14.7 Hz, J = 8.1 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.48–2.44 (dd, J = 14.7 Hz, J = 4.6 Hz, 1H, $C^{\alpha}H_2$ of acyl), 2.38–2.37 (d, J = 5.9 Hz, 2H, $C^{\alpha}H_2$ of acyl), 1.65–1.42 (m, 2H, $C^{\gamma}H_2$ of acyl), 1.30–0.90 (m, 36H, CH_2 of acyl), 0.88–0.86 (t, J = 6.1 Hz, 6H, CH_3 of acyl); Found: C, 70.92; H, 8.94; N, 1.51. Calcd for $C_{57}H_{85}O_{11}N$: C, 71.29; H, 8.92; N, 1.46.

4.9. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-D-seryl)-α-D-glucopyranoside (19a)

To a solution of **17** (300 mg, 312 μmol) and Fmoc-D-Ser(^{t}Bu) (136 mg, 344 μmol) in absolute tetrahydrofuran

(5 mL) were added 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (284 mg, 937 μmol) and *N*-methyl imidazole (249 mL, 3.12 μmol) at room temperature under Ar atmosphere. After stirring for 13 h, the mixture was washed with H_2O and brine, and then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica-gel flash column chromatography (30 g, toluene/AcOEt = 7:1 to 5:1) to give **19a** (349 mg, 85%). ESI-TOF (positive) *m*/*z* = 1348.72 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.66 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.66 (1H, d, *J* = 8.8 Hz, Ser-N*H*), 5.16 (1H, t, *J* = 9.9 Hz, H-3), 5.09 (1H, s, –CO_2–CH_2–Ph), 4.75 (1H, d, *J* = 3.4 Hz, H-1), 4.51–4.48 (6H, m, H-6a, Ser-C$^\alpha$*H*, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.38 (2H, t, *J* = 8.1 Hz, CH_2 of Fmoc), 4.33–4.21 (3H, m, H-2, C*H* of Fmoc, and H-6b), 3.96 (2H, d, *J* = 4.4 Hz, 1-O–CH_2–CO_2–), 3.92–3.79 (4H, m, H-5, C$^\beta$*H* of 2-*O*-, 3-*O*-acyl, and Ser-C$^\beta$*H*a), 3.62–3.55 (2H, m, Ser-C$^\beta$*H*b and H-4), 3.12 (1H, d, *J* = 3.5 Hz, 4-O*H*), 2.63–2.35 (4H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-acyl), 1.16 (9H, s, *t*-Bu), 0.89–0.87 (6H, m, –CH_3 of 2-*O*-, 3-*O*-acyl).

4.10. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-seryl)-α-D-glucopyranoside (19b)

In a manner similar to the synthesis of **19a**, **17** (255 mg, 266 μmol) was condensed with Fmoc-L-Ser(^{t}Bu) (103 mg, 269 μmol) to give **19b** (312 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.65 (1H, d, *J* = 9.2 Hz, 2-N*H*), 5.65 (1H, d, *J* = 8.5 Hz, Ser-N*H*), 5.17 (1H, t, *J* = 10.2 Hz, H-3), 5.09 (2H, s, –CO_2–CH_2–Ph), 4.75 (1H, d, *J* = 3.5 Hz, H-1), 4.57–4.45 (6H, m, H-6a, Ser-C$^\alpha$*H*, and –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.39 (2H, d, *J* = 7.2 Hz, CH_2 of Fmoc), 4.30 (1H, dt, *J* = 14.3, 5.3 Hz, H-2), 4.25–4.19 (2H, m, C*H* of Fmoc and H-6b), 3.95 (2H, d, *J* = 5.2 Hz, 1-O–CH_2–CO_2–), 3.94–3.80 (4H, m, H-5, Ser-C$^\beta$*H*a, and C$^\beta$*H* of 2-*O*-, 3-*O*-acyl), 3.64–3.58 (2H, m, Ser-C$^\beta$*H*b and H-4), 3.07 (1H, d, *J* = 4.2 Hz, 4-O*H*), 2.58–2.30 (4H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-acyl), 1.17 (9H, s, *t*-Bu), 0.88 (6H, t, *J* = 6.8 Hz, –CH_3 of 2-*O*-, 3-*O*-acyl).

4.11. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-*tert*-butyl-D-seryl)-2-deoxy-α-D-glucopyranoside (20a)

To a solution of **19a** (330 mg, 249 μmol) in absolute tetrahydrofuran (10 mL) was added TBD–methyl polystyrene (1.3 g, 2.49 mmol) at room temperature. After stirring for 2.5 d, the resin was filtered off to give crude amine.

To a solution of amine (170 mg, 154 μmol), (*R*)-3-benzyloxytetradecanoic acid (**18a**) (57 mg, 169 μmol), and HOBt (10 mg, 77.9 μmol) in absolute CH_2Cl_2 was added WSCD·HCl (91 mg, 475 μmol) at room temperature under Ar atmosphere. After stirring for 1 d, the mixture was washed with aqueous saturated $NaHCO_3$ and brine, then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica-gel flash column chromatography (10 g, toluene/AcOEt = 4:1) to give **20a** (116 mg, 53%). ESI-TOF (positive) *m*/*z* = 1441.91 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.04 (1H, d, *J* = 8.1 Hz, Ser-N*H*), 6.65 (1H, d, *J* = 9.4 Hz, 2-N*H*), 5.16 (1H, t, *J* = 10.3 Hz, H-3), 5.11 (1H, s, –CO_2–CH_2–Ph), 4.74–4.70 (2H, m, H-1 and Ser-C$^\alpha$*H*), 4.65–4.46 (7H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl, and H-6a), 4.29 (1H, dt, *J* = 13.9, 5.0 Hz, H-2), 4.15 (1H, dd, *J* = 12.0, 2.1 Hz, H-6b), 3.96 (2H, d, *J* = 2.5 Hz, 1-O–CH_2–CO_2–), 3.89–3.74 (5H, m, H-5, C$^\beta$H of 2-*O*-, 3-*O*-, Ser-acyl, and Ser-C$^\beta$*H*a), 3.60–3.52 (2H, m, H-4 and Ser-C$^\beta$*H*b), 3.22 (1H, d, *J* = 3.9 Hz, 4-O*H*), 2.74–2.35 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.07 (9H, t, *J* = 4.3 Hz, *t*-Bu), 0.89–0.87 (9H, m, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.12. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-*tert*-butyl-D-seryl)-α-D-glucopyranoside (20b)

In a manner similar to the synthesis of **20a**, **19b** (142 mg, 107 μmol) was deprotected and acylated with (*R*)-3-benzyloxytetradecanoic acid (**18a**) (62 mg, 186 μmol) to give **20b** (163 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.90 (1H, d, *J* = 8.1 Hz, Ser-N*H*), 6.62 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.16 (1H, dd, *J* = 10.8, 9.3 Hz, H-3), 5.10 (1H, s, –CO_2–CH_2–Ph), 4.74 (2H, dd, *J* = 6.3, 3.4 Hz, H-1 and Ser-C$^\alpha$*H*), 4.58–4.46 (7H, m, H-6a and –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl), 4.29 (1H, ddd, *J* = 11.9, 8.3, 2.5 Hz, H-2), 4.15 (1H, dd, *J* = 12.2, 2.0 Hz, H-6b), 3.95 (2H, d, *J* = 5.1 Hz, 1-O–CH_2–CO_2–), 3.90–3.80 (5H, m, H-5, C$^\beta$*H* of 2-*O*-, 3-*O*-, Ser-acyl and Ser-C$^\beta$*H*a), 3.61–3.52 (2H, m, H-4 and Ser-C$^\beta$*H*b), 3.09 (1H, s, 4-O*H*), 2.63–2.34 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.08 (9H, s, *t*-Bu), 0.88 (9H, dd, *J* = 7.3, 6.4 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.13. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-*tert*-butyl-D-seryl)-α-D-glucopyranoside (21a)

In a manner similar to the synthesis of **20a**, **19a** (119 mg, 108 μmol) was deprotected and acylated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (**18b**) (49 mg, 108 μmol) to give **21a** (77 mg, 52%). ESI-TOF (positive) *m*/*z* = 1563.29 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.68 (1H, d, *J* = 9.3 Hz, 2-N*H*), 6.53 (1H, d, *J* = 8.2 Hz, Ser-N*H*), 5.22–5.12 (2H, m, H-3 and C$^\beta$*H* of Ser-acyl), 5.11 (2H, s, –CO_2–CH_2–Ph), 4.76 (1H, d, *J* = 3.5 Hz, H-1), 4.69 (1H, dt, *J* = 7.9, 3.0 Hz, Ser-C$^\alpha$*H*), 4.58–4.44 (7H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl, and H-6a), 4.28 (1H, dt, *J* = 14.6, 5.1 Hz, H-2), 4.17 (2H, d, *J* = 10.4 Hz, H-6b), 3.97 (2H, d, *J* = 2.0 Hz, 1-O–CH_2–CO_2–), 3.92–3.85 (1H, m, H-5), 3.84–3.77 (3H, m, Ser-C$^\beta$*H*a and C$^\beta$*H* of 2-*O*-, 3-*O*-acyl), 3.62–3.54 (2H, m, H-4 and Ser-C$^\beta H_2$), 3.32 (1H, d, *J* = 3.8 Hz, 4-O*H*), 2.53–2.28 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.14 (9H, s, *t*-Bu), 0.88 (12H, t, *J* = 6.8, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.14. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-*tert*-butyl-L-seryl)-α-D-glucopyranoside (21b)

In a manner similar to the synthesis of **20a**, **19b** was deprotected and acylated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (**18b**) to give **21b** (68 mg, 55%). ESI-TOF (positive) *m*/*z* = 1562.69 [(M+Na)$^+$]; ^{1}H NMR (270 MHz, $CDCl_3$) δ = 6.63 (1H, d, *J* = 9.4 Hz, 2-N*H*), 6.46 (1H, d, *J* = 8.1 Hz, Ser-N*H*), 5.20–5.11 (4H, m, H-3, C$^\beta$*H* of Ser-acyl and –CO_2–CH_2–Ph), 4.75 (1H, d, *J* = 3.5 Hz, H-1), 4.72 (1H, dd, *J* = 6.8, 4.1 Hz, Ser-C$^\alpha$*H*), 4.55–4.45 (5H, m, H-6a and –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.29 (1H, dt, *J* = 14.2, 5.4 Hz, H-2), 4.18 (1H, dd, *J* = 12.1, 2.0 Hz, H-6b), 3.95 (2H, d, *J* = 2.3 Hz, 1-O–CH_2–CO_2–), 3.89–3.80 (4H, m, H-5, C$^\beta$*H* of 2-*O*-, 3-*O*-acyl and Ser-C$^\beta$*Ha*), 3.62–3.54 (2H, m, H-4 and Ser-C$^\beta$*Hb*), 3.05 (1H, d, *J* = 4.3 Hz, 4-O*H*), 2.59–2.25 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.15 (9H, s, *t*-Bu), 0.88 (12H, t, *J* = 6.6 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.15. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-*tert*-butyl-D-seryl)-2-deoxy-α-D-glucopyranoside (22a)

To a solution of **20a** (31 mg, 21.8 μmol) in absolute CH_2Cl_2 (5.0 mL) were added DMAP (21.5 mg, 176 μmol) and ZOBt (52.5 mg, 194 μmol) at room temperature under Ar atmosphere. After stirring for 2.5 d, the mixture was purified by preparative TLC (200 × 100 × 1 mm, toluene/AcOEt = 3:1) to give **22a** (34 mg, quant.). ESI-TOF (positive) *m*/*z* = 1553.93 [(M+H)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.01 (1H, d, *J* = 8.4 Hz, Ser-N*H*), 6.64 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.34 (1H, dd, *J* = 10.6, 9.7 Hz, H-3), 5.10 (2H, s, –CO_2–CH_2–Ph), 5.09 (1H, t, *J* = 6.0 Hz, C*H*H of Z), 4.96 (2H, d, *J* = 6.0 Hz, C*H*H of Z), 4.94 (1H, t, *J* = 10.7 Hz, H-4), 4.76 (1H, dt, *J* = 8.3, 3.1 Hz, Ser-C$^\alpha$*H*), 4.73 (1H, d, *J* = 3.5 Hz, H-1), 4.66–4.32 (7H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl and H-2), 4.20–4.17 (2H, m, H-6), 4.10–4.07 (1H, m, H-5), 3.92 (2H, d, *J* = 3.1 Hz, –CO_2–CH_2–Ph), 3.82–3.73 (4H, m, Ser-C$^\beta$*Ha* and C$^\beta$*H* of 2-*O*-, 3-*O*-, Ser-acyl), 3.53 (1H, dd, *J* = 9.1, 3.2 Hz, Ser-C$^\beta$*Hb*), 2.51–2.29 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.07 (9H, t, *J* = 4.2 Hz, *t*-Bu), 0.88 (9H, t, *J* = 6.7 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.16. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-*tert*-butyl-L-seryl)-2-deoxy-α-D-glucopyranoside (22b)

In a manner similar to the synthesis of **22a**, **20b** (90 mg, 63.4 μmol) was protected by treatment with ZOBt (146 mg, 542 μmol) to give **22b** (86 mg, 88%). ^{1}H NMR (270 MHz, $CDCl_3$) δ = 6.83 (1H, d, *J* = 8.1 Hz, Ser-N*H*), 6.62 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.33 (1H, dd, *J* = 10.7, 9.6 Hz, H-3), 5.09 (2H, d, *J* = 12.2 Hz, C*H*H of Z), 5.09 (2H, s, –CO_2–CH_2–Ph) 4.97–4.88 (1H, m, H-4), 4.96 (1H, d, *J* = 12.2 Hz, C*H*H of Z), 4.74–4.69 (2H, m, H-1 and Ser-C$^\alpha$*H*), 4.55–4.25 (8H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl, H-2 and H-6a), 4.13–3.98 (2H, m, H-6b and H-5), 3.91 (2H, d, *J* = 2.4 Hz, 1-O–CH_2–CO_2–), 3.83–3.72 (4H, m, C$^\beta$*H* of 2-*O*-, 3-*O*-, Ser-acyl and Ser-C$^\beta$*Ha*), 3.49 (1H, dd, *J* = 9.1, 3.5 Hz, Ser-C$^\beta$*Hb*), 2.47–2.29 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.06 (9H, t, *J* = 2.8 Hz, *t*-Bu), 0.88 (9H, t, *J* = 6.6 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.17. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-*tert*-butyl-D-seryl)-α-D-glucopyranoside (23a)

In a manner similar to the synthesis of **22a**, **21a** (58 mg, 37.7 μmol) was protected by treatment with ZOBt (146 mg, 542 μmol) to give **23a** (50 mg, 80%). ESI-TOF (positive) *m*/*z* = 1669.97 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.61 (1H, d, *J* = 8.9 Hz, 2-N*H*), 6.50 (1H, d, *J* = 8.3 Hz, Ser-N*H*), 5.34 (1H, t, *J* = 10.2 Hz, H-3), 5.18 (1H, t, *J* = 6.1 Hz, C$^\beta$*H* of Ser-acyl), 5.10 (1H, d, *J* = 12.1 Hz, C*H*H of Z), 5.10 (2H, s, –CO_2–CH_2–Ph), 4.97 (1H, d, *J* = 12.1 Hz, C*H*H of Z), 4.94 (1H, t, *J* = 9.8 Hz, H-4), 4.75–4.71 (2H, m, H-1 and Ser-C$^\alpha$*H*), 4.51–4.35 (4H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.34 (1H, dt, *J* = 14.6, 5.0 Hz, H-2), 4.23–4.10 (3H, m, H-6 and H-5), 3.92 (2H, dd, *J* = 23.0, 16.6 Hz, 1-O–CH_2–CO_2–), 3.81–3.73 (3H, m, Ser-C$^\beta$*Ha* and C$^\beta$*H* of 2-*O*-, 3-*O*-acyl), 3.55 (1H, dd, *J* = 9.0, 2.9 Hz, Ser-C$^\beta$*Hb*), 2.58–2.23 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.13 (9H, s, *t*-Bu), 0.88 (12H, dd, *J* = 6.9, 6.2 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.18. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-*tert*-butyl-L-seryl)-α-D-glucopyranoside (23b)

In a manner similar to the synthesis of **22a**, **21b** (96 mg, 62.3 μmol) was protected by treatment with ZOBt (252 mg, 935 μmol) to give **23b** (83 mg, 80%). ESI-TOF (positive) *m*/*z* = 1669.18 [(M+Na)$^+$]; ^{1}H NMR (270 Hz, $CDCl_3$) δ = 6.62 (1H, d, *J* = 9.3 Hz, 2-N*H*), 6.46 (1H, d, *J* = 8.3 Hz, Ser-N*H*), 5.34 (1H, dd, *J* = 10.7, 9.6 Hz, H-3), 5.17 (1H, t, *J* = 6.1 Hz, C$^\beta$*H* of Ser-acyl), 5.10 (2H, s, –CO_2–CH_2–Ph), 5.09 (1H, d, *J* = 12.0 Hz, C*H*H of Z), 4.96 (1H, d, *J* = 12.2 Hz, C*H*H of Z), 4.92 (1H, t, *J* = 12.4 Hz, H-4), 4.75 (1H, d, *J* = 3.6 Hz, H-1), 4.68 (1H, dt, *J* = 8.2, 3.1 Hz, Ser-C$^\alpha$*H*), 4.53–4.27 (6H, m, –CH_2–Ph of 2-*O*-, 3-*O*-acyl, H-2 and H-6a), 4.16–4.05 (2H, m, H-5 and H-6a), 3.91 (2H, d, *J* = 2.3 Hz. 1-O–CH_2–CO_2–), 3.85–3.74 (3H, m, C$^\beta$*H* of 2-*O*-, 3-*O*-acyl and Ser-C$^\beta$*H*a), 3.51 (1H, dd, *J* = 9.1, 3.3 Hz, Ser-C$^\beta$*H*b), 2.53–2.23 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 1.12 (9H, s, *t*-Bu), 0.88 (12H, t, *J* = 6.6 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.19. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-D-seryl)-2-deoxy-α-D-glucopyranoside (24a)

The 20% TFA solution in CH_2Cl_2 was prepared by addition of neat TFA (1.0 mL) to absolute CH_2Cl_2 (4.0 mL) and MS4A under Ar atmosphere. To a solution of **22a** (23 mg, 15.2 μmol) in 20% TFA solution (1.0 mL) was added anhydrous anisole (20 μL, 184 μmol) at room temperature under Ar atmosphere. After stirring for 18 h, the mixture was washed with aqueous saturated $NaHCO_3$ and brine, then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica-gel flash column chromatography (10 g, toluene/AcOEt = 2:1) to give **24a** (18.5 mg, 84%). ESI-TOF (positive) m/z = 1497.92 $[(M+H)^+]$; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.00 (1H, d, J = 7.6 Hz, Ser-N*H*), 6.61 (1H, d, J = 9.3 Hz, 2-N*H*), 5.33 (1H, dd, J = 10.6, 9.5 Hz, H-3), 5.09–5.06 (3H, m, –CO_2–CH_2–Ph and C*H*H of Z), 4.94–4.88 (2H, m, C*H*H of Z and H-4), 4.70 (1H, d, J = 3.5 Hz, H-1), 4.68–4.65 (1H, m, Ser-C$^\alpha$*H*), 4.61–4.38 (6H, m, –O–CH_2–Ph of 2-O-, 3-O-, Ser-acyl), 4.33 (1H, ddd, J = 11.8, 8.3, 2.5 Hz, H-2), 4.22 (2H, s, H-6), 4.11 (1H, dt, J = 10.2, 3.2 Hz, H-5), 3.97–3.93 (1H, m, Ser-C$^\beta$*H*a), 3.89–3.72 (4H, m, Ser-C$^\beta$*H*b and C$^\beta$*H* of 2-*O*-, 3-*O*-, Ser-acyl), 3.88 (2H, d, J = 6.0 Hz, 1-O–CH_2–CO_2–), 2.62 (1H, s, Ser-O*H*), 2.51–2.29 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.88 (9H, dd, J = 8.5, 4.8 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.20. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-L-seryl)-2-deoxy-α-D-glucopyranoside (24b)

In a manner similar to the synthesis of **24a**, **22b** (80 mg, 51.5 μmol) was deprotected to give **24b** (70 mg, 91%). ^{1}H NMR (270 MHz, $CDCl_3$) δ = 6.92 (1H, d, J = 8.0 Hz, Ser-N*H*), 6.60 (1H, d, J = 9.3 Hz, 2-N*H*), 5.35 (1H, t, J = 10.2 Hz, H-3), 5.10 (2H, s, –CO_2–CH_2–Ph), 5.03 (1H, d, J = 12.1 Hz, C*H*H of Z), 4.95 (1H, t, J = 9.9 Hz, H-4), 4.87 (1H, d, J = 12.1 Hz, C*H*H of Z), 4.74 (1H, d, J = 3.5 Hz, H-1), 4.69 (1H, dd, J = 6.9, 4.1 Hz, Ser-C$^\alpha$*H*), 4.57–4.27 (8H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl, H-2 and H-6a), 4.20–4.13 (2H, m, Ser-C$^\beta$*H*a and H-5), 3.98 (1H, t, J = 6.1 Hz, H-6b), 3.88 (2H, d, J = 6.1 Hz, 1-O–CH_2–CO_2–), 3.84–3.74 (4H, m, C$^\beta$H of 2-*O*-, 3-*O*-, Ser-acyl and Ser-C$^\beta$*H*b), 2.79 (1H, s, Ser-O*H*), 2.55–2.30 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.88 (9H, t, J = 6.6 Hz, CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.21. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-D-seryl)-α-D-glucopyranoside (25a)

In a manner similar to the synthesis of **24a**, **23a** (42 mg, 25.1 μmol) was deprotected to give **25a** (40 mg, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.63–6.61 (2H, m, 2-N*H* and Ser-N*H*), 5.34 (1H, t, J = 10.1 Hz, H-3), 5.26–5.23 (1H, m, C$^\beta$*H* of Ser-acyl), 5.09 (3H, d, J = 9.8 Hz, –CO_2–CH_2–Ph and C*H*H of Z), 4.94 (1H, d, J = 12.3 Hz, C*H*H of Z), 4.92 (1H, t, J = 9.8 Hz, H-4), 4.75 (1H, d, J = 3.5 Hz, H-1), 4.64–4.60 (1H, m, Ser-C$^\alpha$*H*), 4.52–4.38 (4H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.35 (1H, dt, J = 15.0, 5.6 Hz, H-2), 4.29–4.19 (1H, m, H-6), 4.17–4.14 (1H, m, H-5), 3.94–3.91 (3H, m, Ser-C$^\beta$*H*a and 1-O–CH_2–CO_2–), 3.86–3.72 (3H, m, Ser-C$^\beta$*H*b and C$^\beta$*H* of 2-*O*-, 3-*O*-acyl), 2.89 (1H, s, Ser-O*H*), 2.57–2.23 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.88 (12H, t, J = 6.7 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.22. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-L-seryl)-α-D-glucopyranoside (25b)

In a manner similar to the synthesis of **24a**, **23b** (72 mg, 43.0 μmol) was deprotected to give **25b** (62 mg, 90%). ESI-TOF (positive) m/z = 1639.99 $[(M+Na)^+]$; ^{1}H NMR (270 MHz, $CDCl_3$) δ = 6.60 (1H, d, J = 9.3 Hz, 2-N*H*), 6.54 (1H, d, J = 8.0 Hz, Ser-N*H*), 5.36 (1H, dd, J = 10.7, 9.7 Hz, H-3), 5.20 (1H, t, J = 6.0 Hz, C$^\beta$*H* of Ser-acyl), 5.10 (2H, s, –CO_2–CH_2–Ph), 5.05 (1H, d, J = 12.1 Hz, C*H*H of Z), 4.95 (1H, t, J = 9.9 Hz, H-4), 4.89 (1H, d, J = 12.1 Hz, C*H*H of Z), 4.74 (1H, d, J = 3.6 Hz, H-1), 4.67 (1H, td, J = 5.3, 2.8 Hz, Ser-C$^\alpha$*H*), 4.55–4.28 (6H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl, H-2 and H-6a), 4.21–4.12 (2H, m, Ser-C$^\beta$*H*a and H-5), 4.00–3.96 (1H, m, H-6b), 3.92–3.74 (3H, m, Ser-C$^\beta$*H*b and C$^\beta$*H* of 2-*O*-, 3-*O*-acyl), 3.88 (2H, d, J = 6.8 Hz, 1-O–CH_2–CO_2–), 2.92 (1H, s, Ser-O*H*), 2.55–2.25 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.88 (12H, t, J = 6.6 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.23. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-D-seryl)-2-deoxy-α-D-glucopyranoside (26a)

To a solution of **24a** (30 mg, 20.0 μmol) in absolute CH_2Cl_2 (2.0 mL) were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (24 mg, 100 μmol) and 1*H*-tetrazole (7 mg, 100 μmol) at room temperature under Ar atmosphere. After stirring for 50 min, the mixture was cooled to −20 °C and *m*-chloroperbenzoic acid (20 mg, 30.1 μmol) was added, then stirring was continued for another 1.5 h. The mixture was washed with aqueous saturated $NaHCO_3$ and brine, then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica-gel flash column chromatography (5 g, toluene/AcOEt = 3:1) to give **26a** (29 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.22–7.18 (1H, m, Ser-N*H*), 6.62 (1H, d, J = 9.3 Hz, 2-N*H*), 5.33 (1H, dd, J = 10.6, 9.7 Hz, H-

3), 5.13–5.02 (7H, m, C*H*H of Z and P–O–CH_2–Ph), 4.94–4.89 (3H, m, C*H*H of Z and Ser-C$^{\alpha}H$), 4.67 (1H, d, J = 3.5 Hz, H-1), 4.62–4.32 (9H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Ser-acyl, Ser-C$^{\beta}H_2$ and H-2), 4.29–4.19 (2H, m, H-6), 4.12 (1H, ddd, J = 10.2, 4.6, 2.4 Hz, H-5), 3.91 (2H, s, 1-O–CH_2–CO_2–), 3.85–3.73 (3H, m, C$^{\beta}H$ of 2-*O*-, 3-*O*-, Ser-acyl), 2.51–2.30 (6H, m, C$^{\alpha}H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.91–0.87 (9H, m, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.24. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradeca-noyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-L-seryl)-2-deoxy-α-D-glucopyranoside (26b)

In a manner similar to the synthesis of **26a**, **24b** (65 mg, 43.4 μmol) was phosphorylated with *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (52 mg, 217 μmol) and *m*-chloroperbenzoic acid (44 mg, 260 μmol) to give **26b** (42 mg, 59%).

4.25. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-D-seryl)-α-D-glucopyranoside (27a)

In a manner similar to the synthesis of **26a**, **25a** (32 mg, 19.8 μmol) was phosphorylated with *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (24 mg, 99.9 μmol) and *m*-chloroperbenzoic acid (10 mg, 59.3 μmol) to give **27a** (14 mg, 40%). ESI-TOF (positive) m/z = 1799.09 [(M+H)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.88 (1H, d, J = 7.8 Hz, 2-N*H*), 6.61 (1H, d, J = 9.3 Hz, Ser-N*H*), 5.33 (1H, t, J = 10.1 Hz, H-3), 5.25–5.14 (5H, m, C$^{\beta}H$ of Ser-acyl and P–O–CH_2–Ph), 5.09 (2H, s, –CO_2–CH_2–Ph), 5.07 (1H, d, J = 7.6 Hz, C*H*H of Z), 4.95–4.91 (2H, m, C*H*H of Z and H-4), 4.86–4.82 (1H, m, Ser-C$^{\alpha}H$), 4.70 (1H, d, J = 3.6 Hz, H-1), 4.56–4.30 (8H, m, Ser-C$^{\beta}H_2$, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl H-2 and H-6a), 4.20–4.13 (2H, m, H-6b and H-5), 3.91 (2H, d, J = 3.3 Hz, 1-O–CH_2–CO_2–), 3.81 (1H, t, J = 5.8 Hz, C$^{\beta}H$ of 2-*O*-acyl), 3.74 (1H, t, J = 5.6 Hz, C$^{\beta}$H of 3-*O*-acyl), 2.59–2.26 (8H, m, C$^{\alpha}H_2$ of 2-*O*-, 3-*O*-, Ser-acyl), 0.88 (12H, dd, J = 7.4, 6.3 Hz, –CH_3 of 2-*O*-, 3-*O*-, Ser-acyl).

4.26. Benzyloxycarbonylmethyl 4-*O*-benzyloxycarbonyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-L-seryl)-α-D-glucopyranoside (27b)

In a manner similar to the synthesis of **26a**, **25b** (47 mg, 29.0 μmol) was phosphorylated with *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (35 mg, 145 μmol) and *m*-chloroperbenzoic acid (15 mg, 87.1 μmol) to give **27b** (33 mg, 64%).

4.27. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-*O*-phospho-D-seryl)-2-deoxy-α-D-glucopyranoside (D-Ser(P)1011 (5a))

To a solution of **26a** (14 mg, 8.33 μmol) in distilled THF (1 mL) was added Pd-black (27 mg). The mixture was stirred under 20 kgf/cm^2 of hydrogen at room temperature for 3 days. After removal of the Pd catalyst by filtration, the solution was concentrated with *t*-BuOH and water then lyophilized to give **5a** as a white solid (7.2 mg, 80%). The purity was checked by TLC ($CHCl_3$/MeOH/H_2O = 6:4:1) and ESI-MS ESI-TOF (negative) m/z = 1081.6524 [(M−H)$^-$], 540.3447 [(M−2H)$^{2-}$]; HRMS (ESI-TOF) calcd for $C_{53}H_{97}N_2O_{18}P/2$ ([M−2H]$^{2-}$): 540.3237; found: 540.3239.

4.28. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-*O*-phospho-L-seryl)-2-deoxy-α-D-glucopyranoside (L-Ser(P)1011 (5b))

In a manner similar to the synthesis of **5a**, **26b** (15 mg, 8.93 μmol) was hydrogenated with Pd-black (143 mg, 1.34 mmol) to give **5b** as a white solid (9.7 mg, quant.).

4.29. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-phospho-D-seryl)-2-deoxy-α-D-glucopyranoside (D-Ser(P)2011 (6a))

In a manner similar to the synthesis of **5a**, **27a** (9 mg, 5.00 μmol) was hydrogenated with Pd-black (16 mg, 150 μmol) to give **6a** as a white solid (6.4 mg, 74%).

ESI-TOF (negative) m/z = 1291.5998 [(M−H)$^-$], 645.3690 [(M−2H)$^{2-}$]; HRMS (ESI-TOF) calcd for $C_{67}H_{123}N_2O_{19}P/2$ ([M−2H]$^{2-}$): 645.4229; found: 645.4233.

4.30. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*-phospho-L-seryl)-2-deoxy-α-D-glucopyranoside (L-Ser(P)2011 (6b))

In a manner similar to the synthesis of **5a**, **27b** (18 mg, 10.0 μmol) was hydrogenated with Pd-black (32 mg, 300 μmol) to give **6b** as a white solid (11.5 mg, 89%). ESI-TOF (negative) m/z = 1290.6164 [(M−H)$^-$], 645.2721 [(M−2H)$^{2-}$].

4.31. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-*tert*-butyl-D-seryl)-2-deoxy-α-D-glucopyranoside (28a)

To a solution of **19a** (330 mg, 249 μmol) in absolute tetrahydrofuran (10 mL) was added TBD–methyl polystyrene (1.3 g, 2.49 mmol) at room temperature. After stirring for 2.5 d, the resin was filtered off to give crude amine.

To a solution of amine (94 mg, 85.2 μmol), (*R*)-3-benzyloxytetradecanoic acid (**18a**) (108 mg, 323 μmol), HOBt (5.8 mg, 42.6 μmol), and DMAP (5.2 mg, 42.6 μmol) in absolute CH_2Cl_2 (2.0 mL) was added WSCD·HCl (122 mg, 636 μmol) at room temperature under Ar atmosphere. After stirring for 11 h, the mixture was washed with aqueous saturated $NaHCO_3$ and brine, then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica-gel flash column chromatography (10 g, toluene/AcOEt = 8:1) to give **28a** (80 mg, 61%). ESI-TOF (positive) *m*/*z* = 1737.62 [(M+H)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99 (1H, d, *J* = 8.4 Hz, Ser-N*H*), 6.60 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.32 (1H, t, *J* = 10.2 Hz, H-3), 5.10 (1H, t, *J* = 9.8 Hz, H-4), 5.10 (2H, s, –CO_2–CH_2–Ph), 4.73–4.71 (2H, m, Ser-C$^\alpha$*H* and H-1), 4.65–4.32 (9H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl and H-2), 4.16 (1H, d, *J* = 10.7 Hz, H-6a), 4.07 (1H, dd, *J* = 12.1, 5.3 Hz, H-6b), 4.00–3.98 (1H, m, H-5), 3.93 (2H, s, 1-O–CH_2–CO_2–), 3.77 (5H, m, C$^\beta$*H* of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl and Ser-C$^\beta$*H*a), 3.53 (1H, dd, *J* = 9.1, 3.2 Hz, Ser-C$^\beta$*H*b), 2.51–2.29 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 1.06 (9H, t, *J* = 4.5 Hz, *t*-Bu), 0.88 (12H, t, *J* = 6.6 Hz, –CH_3 of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl).

4.32. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-*tert*-butyl-L-seryl)-2-deoxy-α-D-glucopyranoside (28b)

In a manner similar to the synthesis of **28a**, **19b** was deprotected and acylated with **18a** to give **28b** (47 mg, 55%). ESI-TOF (positive) *m*/*z* = 1759.24 [(M+Na)$^+$].

4.33. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-D-seryl)-2-deoxy-α-D-glucopyranoside (29a)

In a manner similar to the synthesis of **24a**, **28a** (72 mg, 43.0 μmol) was deprotected to give **29a** (51 mg, 83%). ESI-TOF (positive) *m*/*z* = 1702.0438 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99 (1H, d, *J* = 7.6 Hz, Ser-N*H*), 6.57 (1 H, d, *J* = 9.3 Hz, 2-N*H*), 5.32 (1H, t, *J* = 10.2 Hz, H-3), 5.10 (2H, s, –CO_2–CH_2–Ph), 5.08 (1H, t, *J* = 9.0 Hz, H-4), 4.69 (1H, d, *J* = 3.6 Hz, H-1), 4.66–4.62 (1H, m, Ser-C$^\alpha$*H*), 4.61–4.37 (8H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 4.33 (1H, ddd, *J* = 11.8, 8.3, 2.4 Hz, H-1), 4.12 (2H, d, *J* = 2.6 Hz, H-6), 3.99 (1H, dt, *J* = 10.3, 3.2 Hz, H-5), 3.94–3.86 (3H, m, Ser-C$^\beta$*H*a and 1-O–CH_2–CO_2–), 3.85–3.66 (5H, m, Ser-C$^\beta$*H*b and C$^\beta$*H* of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 2.67 (1H, d, *J* = 6.3 Hz, Ser-O*H*), 2.50–2.29 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 0.90–0.86 (12H, m, –CH_3 of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl).

4.34. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-L-seryl)-2-deoxy-α-D-glucopyranoside (29b)

In a manner similar to the synthesis of **24a**, **28b** (35 mg, 20.2 μmol) was deprotected to give **29b** (27 mg, 82%). ESI-TOF (positive) *m*/*z* = 1680.05 [(M+H)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.87 (1H, d, *J* = 8.1 Hz, Ser-N*H*), 6.61 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.34 (1H, t, *J* = 10.2 Hz, H-3), 5.15 (1H, t, *J* = 9.9 Hz, H-4), 5.11 (2H, s, –CO_2–CH_2–Ph), 4.73 (1H, d, *J* = 3.6 Hz, H-1), 4.68–4.65 (1H, m, Ser-C$^\alpha$*H*), 4.58–4.33 (9H, m, -O–CH_2–Ph of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl, H-2), 4.20–4.16 (2H, m, H-6), 3.96 (1H, d, *J* = 10.2 Hz, H-5), 3.88–3.81 (5H, m, 1-O–CH_2–CO_2– and C$^\beta$*H* of 2-*O*-, 3-*O*-, 4-*O*-acyl), 3.76 (2H, dt, *J* = 18.2, 6.3 Hz, Ser-C$^\beta H_2$), 3.64–3.59 (1H, m, C$^\beta$*H* of of Ser-acyl), 3.04 (1H, s, Ser-O*H*), 2.52–2.30 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 0.90–0.85 (12H, m, –CH_3 of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl).

4.35. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-D-seryl)-2-deoxy-α-D-glucopyranoside (30a)

In a manner similar to the synthesis of **26a**, **29a** (37 mg, 22.0 μmol) was phosphorylated with *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (22 mg, 91.2 μmol) and *m*-chloroperbenzoic acid (18 mg, 104 μmol) to give **30a** (20 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.26 (1H, m, Ser-N*H*), 6.59 (1H, d, *J* = 9.3 Hz, 2-N*H*), 5.31 (1H, t, *J* = 10.2 Hz, H-3), 5.12–5.00 (7H, m, P–O–CH_2–Ph, H-4 and –CO_2–CH_2–Ph), 4.89–4.86 (1H, m, Ser-C$^\alpha$*H*), 4.67 (1H, d, *J* = 3.6 Hz, H-1), 4.62–4.32 (11H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl, Ser-C$^\beta H_2$ and H-2), 4.23–4.17 (1H, m, H-6a), 4.14–4.09 (1H, m, H-6b), 4.02 (1H, m, H-5) 3.91 (2H, s, 1-O–CH_2–CO_2–), 3.83–3.66 (4H, m, C$^\beta$*H* of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 2.45–2.33 (4H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 0.92–0.88 (12H, m, –CH_3 of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl).

4.36. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3-λ^5-benzodioxaphosphepin-3-yl)-L-seryl)-2-deoxy-α-D-glucopyranoside (30b)

In a manner similar to the synthesis of **26a**, **29b** (10 mg, 5.95 μmol) was phosphorylated with *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (7.5 mg, 31.4 μmol) and *m*-chloroperbenzoic acid (8.2 mg, 47.5 μmol) to give **30b** (8.9 mg, 81%). ESI-TOF (positive) *m*/*z* = 1863.02 [(M+H)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 (1H, m, Ser-N*H*), 6.62 (1H, d, *J* = 9.4 Hz, 2-N*H*), 5.32 (1H, t, *J* = 10.2 Hz, H-3), 5.17–4.89 (5H, m, H-4 and P–O–CH_2–Ph), 4.85–4.83 (1H, m, Ser-C$^\alpha$*H*), 4.68 (1H, d, *J* = 3.7 Hz, H-1), 4.61–4.34 (11H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl, Ser-C$^\beta H_2$ and H-2), 4.17–4.04 (2H, m, H-6), 4.02–3.98 (1H, m, H-5), 3.92 (2H, s, 1-O–CH_2–CO_2–), 3.90–3.65 (4H, m, C$^\beta$*H* of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 2.52–2.29 (8H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl), 0.88 (12H, t, *J* = 6.8 Hz, –CH_3 of 2-*O*-, 3-*O*-, 4-*O*-, Ser-acyl).

4.37. Carboxymethyl 3,4-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-*O*-phospho-D-seryl)-2-deoxy-α-D-glucopyranoside (D-Ser(P)1111 (7a))

In a manner similar to the synthesis of **5a**, **30a** (14.5 mg, 7.79 μmol) was hydrogenated with Pd-black (25 mg, 234 μmol) to give **7a** as a white solid (6.7 mg, 67%). ESI-TOF (negative) m/z = 1307.6122 [$(M-H)^-$], 653.3762 [$(M-2H)^{2-}$]; HRMS (ESI-TOF) calcd for $C_{67}H_{125}N_2O_{20}P/2$ ([$M-2H]^{2-}$): 653.4204; found: 653.4203.

4.38. Carboxymethyl 3,4-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-*O*-phospho-L-seryl)-2-deoxy-α-D-glucopyranoside (L-Ser(P)1111 (7b))

In a manner similar to the synthesis of **5a**, **30b** (23 mg, 12.3 μmol) was hydrogenated with Pd-black (197 mg, 1.85 mmol) to give **7b** as a white solid (12.6 mg, 79%).

4.39. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-*tert*-butoxycarbonyl-O^4-benzyl-D-aspartyl)-2-deoxy-α-D-glucopyranoside (31a)

To a solution of **17** (41.4 mg, 43.1 μmol) and *N*-*t*-butoxycarbonyl-O^4-benzyl-L-aspartic acid (13.9 mg, 43.1 μmol) in absolute CH_2Cl_2 (0.4 mL) were added DMAP (1.1 mg, 8.6 μmol) and DCC (10.7 mg, 51.7 μmol) at room temperature under Ar atmosphere. After stirring for 1 h, the mixture was washed with aqueous saturated $NaHCO_3$ and brine, then extracted with AcOEt. The organic layer was dried over Na_2SO_4. The mixture was purified by silica-gel flash column chromatography (5 g, $CHCl_3$/acetone = 6:1) to give **31a** as a white solid (42.5 mg, 78%). MALDI-TOF (positive) m/z = 1287.57 [$(M+Na)^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38–7.20 (20H, m, OCH_2Ph) 6.67–6.65 (1H, d, J = 9.5 Hz, 2-N*H*), 5.47-5.45 (1H, d, J = 8.5 Hz, Asp-N*H*), 5.16–5.07 (5H, m, H-3 and –CO_2–CH_2–Ph), 4.74–4.73 (1H, d, J = 3.7 Hz, H-1), 4.58–4.50 (5H, m, Asp-C$^{\alpha}$*H* and –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.41–4.37 (1H, dd, J = 12.0, 4.6 Hz, H-6a), 4.32–4.23 (2H, m, H-2 and H-6b), 4.01–3.97 (1H, d, J = 16.5 Hz, 1-O–CH_2–CO_2–), 3.95–3.91 (1H, d, J = 16.6 Hz, 1-O–CH_2–CO_2–), 3.89–3.80 (3H, m, H-5 and C$^{\beta}$*H* of 2-*O*-, 3-*O*-acyl), 3.57–3.52 (1H, ddd, J = 9.3, 9.3, 4.1 Hz, H-4), 3.12–3.10 (1H, d, J = 4.1 Hz, 4-O*H*), 3.03–2.98 (1H, dd, J = 17.0, 4.6 Hz, Asp-C$^{\beta}H_2$), 2.90–2.85 (1H, dd, J = 17.0, 4.6 Hz, Asp-C$^{\beta}H_2$), 2.64–2.36 (4H, m, C$^{\alpha}H_2$ of 2-*O*-, 3-*O*-acyl), 1.59–1.17 (49 H, m, CH_3 of Boc and CH_2 of 2-*O*-, 3-*O*-acyl), 0.90–0.84 (6H, t, –CH_3 of 2-*O*-, 3-*O*-acyl).

4.40. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-*tert*-butoxycarbonyl-O^4-benzyl-L-aspartyl)-2-deoxy-α-D-glucopyranoside (31b)

In a manner similar to the synthesis of **31a**, **17** (100 mg, 104 μmol) was condensed with *N*-*tert*-butoxycarbonyl-O^4-benzyl-L-aspartic acid (34 mg, 105 μmol) to give **31b** as a white solid (128 mg, 97%). ESI-TOF (positive) m/z = 1287.51 [$(M+Na)^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.67 (1H, d, J = 9.4 Hz, 2-N*H*), 5.45 (1H, d, J = 7.9 Hz, Asp-N*H*), 5.17–5.05 (5H, m, H-3 and –CO_2–CH_2–Ph), 4.71 (1H, d, J = 3.6 Hz, H-1), 4.60–4.58 (1H, m, Asp-C$^{\alpha}$*H*), 4.50 (4H, dd, J = 14.0, 11.6 Hz, –O–CH_2–Ph of 2-*O*-, 3-*O*-acyl), 4.41 (1H, dd, J = 12.0, 4.2 Hz, H-6a), 4.30–4.22 (2H, m, H-2 and H-6b), 3.96 (2H, d, J = 1.8 Hz, 1-O–CH_2–CO_2–), 3.92–3.79 (3H, m, H-5 and C$^{\beta}$*H* of 2-*O*-, 3-*O*-acyl), 3.54 (1H, td, J = 9.6, 4.5 Hz, H-4), 3.09 (1H, d, J = 4.5 Hz, 4-O*H*), 2.96 (2H, ddd, J = 63.1, 17.1, 4.4 Hz, Asp-C$^{\beta}H_2$), 3.60–2.35 (4H, m, C$^{\alpha}H_2$ of 2-*O*-, 3-*O*-acyl), 0.90–0.86 (6H, m, –CH_3 of 2-*O*-, 3-*O*-acyl).

4.41. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-O^4-benzyl-D-aspartyl)-2-deoxy-α-D-glucopyranoside (32a)

To a solution of **31a** (40.8 mg, 32.2 μmol) in absolute CH_2Cl_2 (0.5 mL) was added TFA (125 μL) at 0 °C under Ar atmosphere. After stirring for 20 min at room temperature, the mixture was quenched with aqueous saturated $NaHCO_3$ and brine, then extracted with AcOEt. The organic layer was dried over Na_2SO_4, and concentrated in vacuo to give crude amine.

To the solution of crude amine (17.7 mg, 15.1 μmol) in absolute $CHCl_3$ (0.2 mL), (*R*)-3-benzyloxytetradecanoic acid (**18a**) (8.8 mg, 26.3 μmol), and HOBt (4.1 mg, 30.3 μmol) was added WSCD·HCl (5.8 mg, 30.3 μmol) at room temperature under Ar atmosphere. After stirring overnight, the mixture was purified with preparative TLC (200 × 100 × 0.5 mm, $CHCl_3$/acetone = 15:1) to give **32a** as a white solid (13.9 mg, 62%). ESI-TOF (positive) m/z = 1503.11 [$(M+Na)^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38–7.20 (m, 25H, OCH_2Ph), 7.12–7.10 (d, J = 7.6 Hz, 1H, Asp-N*H*), 6.66–6.64 (d, J = 9.5 Hz, 1H, 2-N*H*), 5.15–5.10 (dd, J = 11.0 Hz, J = 9.5 Hz, 1H, H-3), 5.09–5.03 (m, 4H, $COOCH_2$Ph), 4.87–4.83 (ddd, J = 4.6 Hz, J = 5.2 Hz, J = 7.9 Hz, 1H, Asp-C$^{\alpha}$*H*), 4.71–4.70 (d, J = 3.7 Hz, 1H, H-1), 4.58–4.47 (m, 6H, OCH_2Ph), 4.39–4.35 (dd, J = 5.2 Hz, J = 11.9 Hz, 1H, H-6a), 4.31–4.25 (ddd, J = 3.7 Hz, J = 10.7 Hz, J = 9.5 Hz, 1H, H-2), 4.23–4.20 (dd, J = 2.1 Hz, J = 11.9 Hz, 1H, H-6b), 3.99–3.94 (d, J = 16.8 Hz, 1H, C*H*H of 1-O–CH_2–CO_2–), 3.94–3.89 (d, J = 16.8 Hz, 1H, C*H*H of 1-O–CH_2–CO_2–), 3.88–3.77 (m, 4H, C$^{\beta}$*H* of acyl), 3.53–3.48 (dd, J = 9.5 Hz, J = 10.7 Hz, 1H, H-4), 3.08 (s, 1H, 4-O*H*), 2.98–2.93 (dd, J = 17.1 Hz, J = 4.9 Hz, 1H, Asp-C$^{\beta}H_2$), 2.90–2.85 (dd, J = 17.0 Hz, J = 4.6 Hz, 1H, Asp-C$^{\beta}H_2$), 2.63–2.36 (m, 3H, C$^{\alpha}H_2$ of acyl), 1.59–1.25 (m, 60H, CH_2 of acyl), 0.90–0.86 (t, J = 7.0 Hz, 9H, CH_3 of acyl).

4.42. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-benzyloxytetradecanoyl)-O^4-benzyl-L-aspartyl)-2-deoxy-α-D-glucopyranoside (32b)

In a manner similar to the synthesis of **32a**, **31b** (74 mg, 58.5 μmol) was dissolved in 50% CH_2Cl_2 solution of TFA to give crude amine (62 mg).

The crude amine (27.8 mg, 23.9 μmol) was condensed with (*R*)-3-benzyloxytetradecanoic acid (**18a**) (8.0 mg, 23.9 μmol) to give **32b** (27.3 mg, 77%). ESI-TOF (positive) *m/z* = 1503.11 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.04 (1H, d, *J* = 8.0 Hz, Asp-N*H*), 6.65 (1H, d, *J* = 9.4 Hz, 2-N*H*), 5.16–4.96 (5H, m, H-3, –O–CH_2–Ph), 4.90–4.85 (1H, m, Asp-C$^\alpha$*H*), 4.69 (1H, d, *J* = 3.6 Hz, H-1), 4.52–4.43 (6H, m, –O–CH_2–Ph of 2-*O*-, 3-*O*-, Asp-acyl), 4.37 (1H, dd, *J* = 12.0, 4.8 Hz, H-6a), 4.30–4.20 (2H, m, H-2 and H-6b), 3.95 (2H, s, 1-O–CH_2–CO_2–), 3.90–3.73 (3H, m, H-5 and C$^\beta$*H* of 2-*O*-, 3-*O*-, Asp-acyl), 3.52 (1H, td, *J* = 9.7, 3.6 Hz, H-4), 3.10 (1H, d, *J* = 4.0 Hz, 4-O*H*), 2.92 (2H, ddd, *J* = 66.9, 17.1, 4.7 Hz, Asp-C$^\beta H_2$), 2.62–2.36 (6H, m, C$^\alpha H_2$ of 2-*O*-, 3-*O*-, Asp-acyl), 0.88 (9H, dd, *J* = 7.0, 6.3 Hz, –CH_3 of 2-*O*-, 3-*O*-, Asp-acyl).

4.43. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*^{4}-benzyl-D-aspartyl)-α-D-glucopyranoside (33a)

In a manner similar to the synthesis of **32a**, **31a** (40.8 mg, 32.2 μmol) was dissolved in absolute CH_2Cl_2 (0.5 mL) and TFA (125 μL) to give crude amine.

The crude amine (17.7 mg, 15.1 μmol) was condensed with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (**18b**) (13.5 mg, 29.7 μmol) to give **33a** (27.3 mg, 77%). MALDI-TOF (positive) *m/z* = 1623.07 [(M+Na)$^+$]; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73–7.21 (m, 20H, OCH_2*Ph*), 6.72–6.70 (d, *J* = 7.9 Hz, 1H, Asp-N*H*), 6.68–6.66 (d, *J* = 9.5 Hz, 1H, N*H*), 5.16–5.10 (m, 6H, H-3,–CO_2CH_2Ph), 4.86–4.82 (ddd, *J* = 4.6 Hz, *J* = 4.9 Hz, *J* = 7.9 Hz, 1H, Asp-C$^\alpha$*H*), 4.73–4.73 (d, *J* = 3.7 Hz, 1H, H-1), 4.51–4.50 (m, 4H, OCH_2Ph of 2-*O*-, 3-*O*-acyl), 4.38–4.34 (dd, *J* = 4.9 Hz, *J* = 12.2 Hz, 1H, H-6a), 4.31–4.25 (m, 2H, H-2, H-6b), 4.00–3.96 (d, *J* = 16.8 Hz, 1H, C*H* of 1-O–CH_2–CO_2–), 3.95–3.91 (d, *J* = 16.8 Hz, 1H, C*H* of 1-O–CH_2–CO_2–), 3.89–3.80 (m, 3H, C$^\beta$*H* of acyl, H-5), 3.56–3.51 (dd, *J* = 9.5 Hz, *J* = 9.5 Hz, 1H, H-4), 3.04–2.99 (dd, *J* = 17.4 Hz, *J* = 4.9 Hz, 1H, Asp-C$^\beta H_2$), 2.92–2.86 (dd, *J* = 17.0 Hz, *J* = 4.6 Hz, 1H, Asp-C$^\beta H_2$), 2.65–2.39 (m, 4H, C$^\alpha H_2$ of of 2-*O*-, 3-*O*-acyl), 2.30–2.26 (t, *J* = 7.9 Hz, 2H, C$^\alpha H_2$ of 2-*O*-, 3-*O*-acyl), 1.77–1.25 (m, 82H, CH_2 of 2-*O*-, 3-*O*- acyl), 0.90–0.86 (t, *J* = 7.0 Hz, 12H, –CH_3 of 2-*O*-, 3-*O*-, Asp-acyl).

4.44. Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-*O*^{4}-benzyl-D-aspartyl)-α-D-glucopyranoside (33b)

In a manner similar to the synthesis of **32a**, **31b** (74 mg, 58.5 μmol) was dissolved in 50% CH_2Cl_2 solution of TFA to give crude amine (62 mg).

The crude amine (36 mg, 30.9 μmol) was condensed with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (**18b**) (14.0 mg, 30.9 μmol) to give **33a** (31 mg, 64%). ESI-TOF (positive) *m/z* = 1624.70 [(M+Na)$^+$].

4.45. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-D-aspartyl)-2-deoxy-α-D-glucopyranoside (D-Asp1011 (8a))

To a solution of **32a** (7.63 mg, 5.14 μmol) in distilled THF (0.2 mL) was added Pd-black (10.6 mg). The mixture was stirred under 7 kgf/cm^2 of hydrogen at room temperature overnight. After removal of the Pd catalyst by filtration, the solution was concentrated in vacuo. The residue was purified by liquid–liquid partition column chromatography (Sephadex®LH-20, 5 g; solvent, $CHCl_3$/MeOH/H_2O/*i*-PrOH = 8:12:9:1), and then added distilled water and *t*-BuOH. The suspension was lyophilized to give 8**a** as a white powder. The purity was checked by TLC ($CHCl_3$/MeOH/H_2O = 6:4:1) and ESI-MS ESI-TOF (negative) *m/z* = 1030.6 [(M−H)$^-$], 514.3 [(M−2H)$^{2-}$].

4.46. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-hydroxytetradecanoyl)-D-aspartyl)-2-deoxy-α-L-glucopyranoside (L-Asp1011 (8b))

To a solution of **32b** (32 mg, 21.6 μmol) in distilled THF (2 mL) was added Pd-black (69 mg, 648 μmol). The mixture was stirred under 20 kgf/cm^2 of hydrogen at room temperature for 3 days. After removal of the Pd catalyst by filtration, the solution was concentrated with *t*-BuOH and water then lyophilized to give **8b** as a white solid (18 mg, 82%). The purity was checked by TLC ($CHCl_3$/MeOH/H_2O = 6:4:1) and ESI-MS ESI-TOF (negative) *m/z* = 1029.6400 [(M−H)$^-$], 514.3377 [(M−2H)$^{2-}$].

4.47. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-D-aspartyl)-2-deoxy-α-D-glucopyranoside (D-Asp2011 (9a))

To a solution of **33a** (9.67 mg, 6.14 μmol) in distilled THF (0.2 mL) was added Pd-black (9.2 mg). The mixture was stirred under 7 kgf/cm^2 of hydrogen at room temperature overnight. After removal of the Pd catalyst by filtration, the solution was concentrated in vacuo. The residue was added distilled water and *t*-BuOH, and the suspension was lyophilized to give **9a** as a white powder (7.82 mg). The purity was checked by TLC ($CHCl_3$/MeOH/H_2O = 6:4:1) and ESI-MS ESI-TOF (negative) *m/z* = 1239.90 [(M−H)$^-$], 619.5 [(M−2H)$^{2-}$].

4.48. Carboxymethyl 3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-L-aspartyl)-2-deoxy-α-L-glucopyranoside (L-Asp2011 (9b))

In a manner similar to the synthesis of **8b**, **33b** (23 mg, 14.4 μmol) was hydrogenated with Pd-black (46 mg, 431 μmol) to give **9b** as a white solid (18 mg, quant.). ESI-TOF (negative) *m/z* = 1239.8854 [(M−H)$^-$], 619.4328 [(M−2H)$^{2-}$].

4.49. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-O^4-benzyl-D-aspartyl)-α-D-glucopyranoside (34a)

Compound **31a** (56 mg, 44.2 μmol) was dissolved in 50% TFA solution in CH_2Cl_2 (2 mL) at room temperature. After being stirred for 11 h, the mixture was washed with saturated aqueous $NaHCO_3$ and NaCl, then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude amine (51 mg).

To the solution of crude amine in absolute CH_2Cl_2 (2 mL) and (*R*)-3-benzyloxytetradecanoic acid (**18a**) (72 mg, 214 μmol) were added HOBt (6 mg, 42.9 μmol), DMAP (5 mg, 40.9 μmol) and WSCD·HCl (99 mg, 515 μmol) at room temperature under Ar atmosphere. After stirring for 16 h, the mixture was purified by silica-gel flash column chromatography (5 g, toluene/AcOEt = 10:1) to give **34a** (37 mg, 47%).

4.50. Benzyloxycarbonylmethyl 3,4-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-6-*O*-(*N*-((*R*)-3-(tetradecanoyloxy)tetradecanoyl)-O^4-benzyl-L-aspartyl)-α-D-glucopyranoside (34b)

In a manner similar to the synthesis of **34a**, **31b** (74 mg, 58.5 μmol) was dissolved in 50% CH_2Cl_2 solution of TFA to give crude amine (62 mg).

The crude amine (28 mg, 24.0 μmol) was condensed with (*R*)-3-benzyloxytetradecanoic acid (**18a**) (20 mg, 60.1 μmol) to give **34b** (24 mg, 56%). ESI-TOF (positive) *m*/*z* = 1798.5462 [$(M+H)^+$], 899.8038 [$(M+2H)^{2+}$].

4.51. Carboxymethyl 3,4-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-(*R*)-3-hydroxytetradecanoyl-D-aspartyl)-2-deoxy-α-L-glucopyranoside (D-Asp1111 (10a))

In a manner similar to the synthesis of **8b**, **34a** (13.5 mg, 7.51 μmol) was hydrogenated with Pd-black (24 mg, 225 μmol) to give **10a** as a white solid (9 mg, 96%). ESI-TOF (negative) *m*/*z* = 1255.8784 [$(M-H)^-$], 627.4290 [$(M-2H)^{2-}$].

4.52. Carboxymethyl 3,4-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-(*N*-(*R*)-3-hydroxytetradecanoyl-L-aspartyl)-2-deoxy-α-L-glucopyranoside (L-Asp1111 (10b))

In a manner similar to the synthesis of **10a**, **34b** (12 mg, 6.67 μmol) was hydrogenated with Pd-black (22 mg, 207 μmol) to give **10b** as a white solid (7.8 mg, 98%). ESI-TOF (negative) *m*/*z* = 1255.9747 [$(M-H)^-$].

Acknowledgments

We are grateful to Prof. T. Tamura and Ms. K. Aoyama at Hyogo medical college for their assistance in the cytokine induction assay. We also thank Dr. T. Fujita from Suntory Institute for Bioorganic Research for MS measurements, and Mr. S. Adachi for NMR measurements, and Ms. K. Hayashi and Ms. T. Ikeuchi for elemental analysis at Osaka University. The present work was financially supported in part by Grants-in Aid for Scientific Research (No. 17035050, No. 17310128) from the Japan Society for the Promotion of Science, grants from Suntory Institute for Bioorganic Research (SUNBOR Grant), the Houansha Foundation, and Hayashi Memorial Foundation for Female Natural Scientists (Y.F.).

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